



ANNUAL REPORT

**Division of Intramural Research Programs
National Institute of Mental Health**

October 1, 1983 - September 30, 1984

**VOLUME II
INDIVIDUAL PROJECT REPORTS**

**U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Alcohol, Drug Abuse, and Mental Health Administration
National Institute of Mental Health
Division of Intramural Research Programs**

ANNUAL REPORT

DIVISION OF INTRAMURAL RESEARCH PROGRAMS

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VOLUME II

INDIVIDUAL PROJECT REPORTS

ANNUAL REPORT
DIVISION OF INTRAMURAL RESEARCH PROGRAMS

NATIONAL INSTITUTE OF MENTAL HEALTH

October 1, 1983 - September 30, 1984

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NATIONAL INSTITUTE OF MENTAL HEALTH

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Z01MH00071	Z01MH00382	Z01MH00871
Z01MH00072	Z01MH00388	Z01MH00881
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RESEARCH PROJECT SERIAL NUMBER LISTING (Cont.)

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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00092-10 BP

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Central Amines and Aggression, Suicide, and Alcoholism

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Gerald L. Brown, M.D., Medical Officer, BPB, NIMH

Dr. Frederick K. Goodwin, Scientific Director, IRP, NIMH; Cmdr. Peter F. Goyer, M.D., Dept. of Psychiatry, Portsmouth Naval Medical Center; Dr. Robert M. Post, Chief, Biological Psychiatry Branch, NIMH; Dr. Markku Linnoila, Chief, Lab. of Clinical Studies, NIAAA; Captain O.L. Royal, M.C., USN, National Medical Center

COOPERATING UNITS (if any)

Intramural Research Program, NIMH
Laboratory of Clinical Studies, NIAAA; Department of Psychiatry,
National Naval Medical Center; Portsmouth Naval Medical Center

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

1.0

PROFESSIONAL:

.70

OTHER:

.30

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The National Institute of Mental Health (NIMH), both separately and together with the National Naval Medical Center, is studying central amine metabolites in the cerebrospinal fluid (CSF) of psychiatric patients. Results to date indicate that aggression and anti-social behavior are inversely correlated with CSF 5-hydroxyindoleacetic acid (5HIAA). Low CSF 5HIAA is also associated with suicidal history; suicidal history is similarly associated with a history of aggressive, anti-social behavior. Findings have been largely replicated in two separate populations. Alcoholics have decreased CSF 5HIAA during abstinence. Disulfiram (Antabuse) appears to lower CSF homovanillic acid (HVA) and appears to increase serum norepinephrine (NE); low CSF dopamine-beta-hydroxylase (DBH), low platelet monoamine oxidase (MAO), low plasma amine oxidase (AO), and high red-cell catechol-0-methyl transferase (COMT) are related to adverse reactions to disulfiram. CSF DBH is inversely related to significant deviations in certain personality measures on the MMPI; CSF 5HIAA is inversely related to the Pd scale. A trivariate relationship exists between history of aggression, history of suicidal behavior, and lower CSF 5HIAA.

Project Description:

Objectives: Evidence obtained in recent years indicates that epinephrine, norepinephrine (NE), dopamine, serotonin (5HT), acetylcholine, and gamma-aminobutyric acid (GABA), among others, act as neurotransmitters and/or neuromodulators of the central nervous system (CNS). Although considerable indirect pharmacologic evidence has linked these amine systems with psychiatric illness (particularly affective illness and schizophrenia), the relative lack of direct data in man has limited the assessment of linkages to improved diagnosis, though our work raises the possibility that searching for interrelationships between central biochemical functioning and repeated behavioral patterns may be more fruitful than searching for traditional diagnostic specificity of biochemical findings. In any case, further confirmation of relationships between central biochemistry and behavior could lead to more specific pharmacological treatments. Direct data from man can be immensely valuable in making use of the massive data from animals and assessing the differences and similarities between man and animals. There has been a relative dearth of data on central neurochemical function in the various personality disorders -- a rather striking deficit in our knowledge considering the evidence suggesting that some personality disorders, particularly those involving criminality, have patterns of a genetic component. Furthermore, certain patterns of behavior often seen within personality disorders; i.e., depression, alcoholism, and suicide; also appear to have genetic components. Data from animals suggests a relationship between aggressive behavior and neurotransmitters. Furthermore, this project is intended to contribute to a growing body of studies in human alcoholism. A purpose of this project is to extend the studies of central amine turnover into larger and more diverse populations of psychiatric patients and to assess behavioral-biochemical relationships not limited to specific diagnostic categories. Dr. Frederick Goodwin continues to provide current scientific supervision on this multi-faceted project.

Methods Employed: Independent studies are both a separate effort of NIMH and a joint effort with the National Naval Medical Center, in Bethesda, MD and the Portsmouth Naval Medical Center in Portsmouth, VA. Two study groups consisted entirely of military, active duty, inpatient males of normal intelligence; the first study was comprised of 26 subjects and the second, 12. More patients were not available for the second study. The two groups were of the same age range (17 to 32 years) and of a similar mean age (mean \pm SD = 22.1 \pm 3.6 and 22.0 \pm 5.2, respectively). Height was unavailable in the first study, but ranged from 68 to 73 inches in the second study (70.6 \pm 1.4). All study subjects were unpaid volunteers from whom informed consent was obtained. Patients were excluded from the two studies if medical disorders were present or if there was evidence of past or current primary affective disorder or schizophrenia, or if other than transient organic brain syndrome had ever been observed. An important clinical difference between the two groups, however, was that any presence or history of psychotic symptomatology was a basis for exclusion from the first study group; whereas a history of Brief, Reactive Psychosis (DSM-III, No. 298.80) as secondary diagnosis was present in four of the second study group and two others had had episodes of severe withdrawal sufficient to meet the criteria for Schizoid Personality Disorder (DSM-III, No. 301.20) as a secondary diagnosis. Clinical diagnoses and clinical history assessments were made independently of biochemical investigations. Further exclusions criteria were the ingestion of any drug,

prescribed or illicit, within ten days of a scheduled lumbar puncture (LP) and heavy use of alcohol (a score of greater than 6 on the Michigan Alcoholism Screening Test [MAST]). Alcoholic study groups were somewhat older and did score greater than 6 on the MAST. They did not have significant histories of medical, affective, schizophrenic, or organic disorders. Material available for evaluating each patient included full psychiatric/medical history, physical examination, and job performance assessments. Since a purpose of admission was evaluation of suitability for further military service, emphasis was given to a life history of aggression, particularly in response to authority. The categories of behavior used to determine aggression history, its scoring, its reliability, and its use in a normal age- and sex-matched control group have been described in detail in published studies. In addition, the Buss-Durkee Inventory (BDI) for aggression and the Minnesota Multiphasic Personality Inventory (MMPI) have been used. Individual items of the psychopathic deviate (Pd) scale of the MMPI approximate behaviors reflected in the life history of aggression measure. The use of standardized personality assessment instruments should facilitate attempts at further replication. All evaluative and behavioral data were collected, scored, and analyzed independently of the biochemical data.

Cerebrospinal fluid (CSF) was obtained from study group subjects following the procedures developed and revised at NIH and elsewhere. Assay details are described in the published studies. Other studies in conjunction with pharmacological interventions have further provided knowledge of functional brain chemistry in relationship to behavior, diagnosis, and personality.

Currently, new protocols within the Biological Psychiatry Branch and in collaboration with Dr. Robert M. Post are being developed to characterize more clearly those patients who are at risk for aggressive and suicidal behavior. In brief, subjects will have their indoleamine metabolism assessed directly and indirectly in several ways; i.e., repeated LP's, tritiated imipramine binding and 5HT in platelets; a fenfluramine challenge; and oral tryptophan pharmacokinetics. In addition, glucose tolerance testing and chromosomal assessment will be done.

Major Past Findings: Initial results from personality disorders with problems secondary to poor impulse control, high levels of anger-hostility, and poor judgment indicated that aggressive behavior is inversely correlated with 5-hydroxyindoleacetic acid (5HIAA) and positively correlated with 3-methoxy-4-hydroxyphenylglycol (MHPG). Personality disorders have shown no significant difference in CSF cyclic 3',5'-adenosine monophosphate (c-AMP) from neurological patients with non-CNS disorders or from depressive, manic, and schizophrenic patients. Aggressive behavior was positively correlated with c-AMP and c-GMP in one group but not in a second. Those who were administratively discharged from the Service and those with history of suicidal attempts had lower CSF 5HIAA and higher MHPG, c-AMP, and c-GMP. Borderline personalities (DSM-III) show an inverse relationship between CSF 5HIAA and the Pd scale, as well as a history of aggressive behavior; neither the MHPG relationships nor the cyclic nucleotide relationships were replicated. This study of c-AMP and c-GMP in borderlines has not yet been published. The trends are the same as those seen in the first study. Some of the differences between studies that may account for the non-replication of the MHPG and cyclic nucleotide findings are differences in

diagnoses and homogeneity of intra-group behavioral patterns, smaller numbers of patients in the second study, and later refinements in assay methods. A trivariate relationship between a history of aggression, history of suicidal behavior, and lower CSF 5HIAA is readily apparent.

Of further interest is the initiation of new protocols (above) and the assessment of patients who are accused of murder and have a history of impulsive behavior. As our experience accumulates, the aggressive variable that appears to be most likely associated with lower CSF 5HIAA is that characterized by lability of affect, history of repeated impulsivity, and explosiveness. Similarly, our experience and that of others appears to indicate that suicidality associated with aggressivity is most likely to be associated with reduced levels of CSF 5HIAA. We intend to study individuals with histories of repeated suicidal behavior in themselves and their families; similarly, our experience and that of others appears to indicate that suicidality associated with aggressivity is most likely to be associated with reduced levels of CSF 5HIAA. Pilot trials of the new protocols have been completed but results are too preliminary to be reported at this time. Further collaboration with Dr. Goyer, USN involves assessment of suicidality, ³H-IMI, 5HT, and MAO in early teenagers, but no results are yet available.

Alcoholics do not differ from personality disorders in CSF HVA. However, mean CSF 5HIAA is higher in the intoxication-withdrawal stage and decreases over time in abstinence to reach a mean level not differing from that of personality disorders. Although CSF HVA levels do not change post-intoxication-withdrawal, these levels are depressed by disulfiram (Antabuse), a dopamine-beta-hydroxylase (DBH) inhibitor. Disulfiram use also correlates with an increase in serum NE. Mean serum DBH in alcoholics versus normal controls was lower, blood pressure was higher, and serum NE was not different. Disulfiram is also associated with an increase in cholesterol in alcoholics. Lower CSF DBH is correlated with increasing psychopathology, as measured by the MMPI, and lower CSF DBH is associated with disulfiram-induced psychoses. Furthermore, low platelet monoamine oxidase (MAO), low amine oxidase (AO), and elevated erythrocyte catechol-O-methyltransferase (COMT) are associated with disulfiram-induced psychoses. New studies show that neither clinical depression nor aggressive behavior in this group of early to mid-stage alcoholics can be associated with alcoholism; nor can improvement in depression or anxiety ratings of hospitalized alcoholics be attributed to disulfiram.

The above represents both studies that have been earlier published, those in press, and those in preparation. Continued collaboration with Dr. Linnoila of NIAAA involves the new protocols on aggression and suicidality, as well as collaboration on alcoholics to be admitted to NIAAA. With regard to both groups, chromosomal studies and serum electrophoresis studies are underway, but results are preliminary.

New Findings: A military male found guilty of violent murder, with a past history of several suicidal attempts was found to have the lowest level of CSF 5HIAA yet measured by our group; he also had a hypoglycemic response to a glucose tolerance test (GTT). In that aggressive behavior has been shown in animals to be associated with lower GABA, new studies of CSF GABA, both free and bound, are being analyzed in the borderline group of patients.

Though CSF GABA is lower in the more aggressive patients and in those with histories of suicidal behavior, neither difference reaches the $<.05$ level of significance.

Further analyses of previous studies indicate that these individuals as anti-social and explosive (DSM-III) have the lowest levels of CSF 5HIAA; furthermore the MMPI profile of 42, 48, and 49 with high F scale scores are most closely associated with low CSF 5HIAA. The only Buss Durkee Inventory (BDI) category that has a significant inverse relationship with CSF 5HIAA is "irritability". While total BDI scores and PD T scores do correlate significantly with a life history of aggression, the BDI appears to measure a number of aspects of aggressive thoughts and attitudes as well as behavior, but this scale appears to be a less useful instrument to relate to CSF 5HIAA levels. Attempts are now being made to follow the later life course of the original patients.

Significance to Mental Health Research: CNS functioning is greatly understudied in some major groups of psychiatric patients, viz. personality disorders, alcoholics, and borderlines. Studies of animal models, as well as Gilles de la Tourette syndrome, hyperactive children, and prisoners suggest a relationship between central neurotransmitter systems and aggressive behavior. Human suicidal behavior has an enormous public health and social significance and, previously, had largely been studied from a psychological and sociological point of view. These studies lead to the possibility of identifying those at risk for anti-social and suicidal behaviors and possibly altering these behaviors through neuropharmacological adjuncts to management of the psychiatric and/or behavioral problems. The neurobiological aspects of alcoholism, either predisposing, concomitant, or resultant, are of timely significance as alcoholism is a prevalent problem. Also, drug-free personality disorders may serve as a useful comparison group for biological studies of other psychiatric disorders.

Proposed Course of Project: The preparation for this project began in January 1973. The approval processes, both in terms of scientific merit and the protection of rights of patients, were completed in July 1974. The first lumbar puncture was performed in September 1974. The progress of the project has been submitted to the Navy for reapproval each March and has now been terminated with regard to obtaining new subjects. We believe this collaboration continues to be of mutual benefit to NIMH and NNMC. There is still some neurochemical, behavioral, and psychological data to be analyzed and reported from the patients who have participated in these studies as well as the first attempts of a follow-up. Additionally, new protocols of a similar nature are being prepared, as described above, to continue this work within the BPP.

Publications:

Brown, G.L., and Goodwin, F.K.: Aggression, adolescence and psychobiology. In Keith, C.R. (Ed.): The Aggressive Adolescent: Clinical Perspectives. New York, McMillan, 1984, pp. 63-95.

Goyer, P.F., Brown, G.L., Minichiello, M.D., and Major, L.F.: Mood-altering effects of disulfiram (Antabuse) in alcoholics. J. Stud. Alcohol, 45 (3):209-213, 1984.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00100-09 BP

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biobehavioral Aspects in Childhood and Adolescent Mental Illness

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Gerald L. Brown, M.D., Medical Officer, BPB, NIMH

Dr. Michael H. Ebert, Chairman, Department of Psychiatry, Vanderbilt University;
 Dr. Christy L. Ludlow, Research Speech Pathologist, Communicative Disorders
 Program, NINCDS; Dr. Judith Rapoport, Chief, Child Psychiatry, NIMH; Dr. Alan J.
 Zametkin, Staff Investigator, University of Auckland, New Zealand; NIMH; Dr.
 Robert M. Post, Chief, Biological Psychiatry Branch, NIMH

COOPERATING UNITS (if any)

Communicative Disorders Program, NINCDS; Child Psychiatry Branch, NIMH
 University of Auckland, New Zealand

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

4.0

PROFESSIONAL:

1.0

OTHER:

3.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

An inpatient program with selected overnight stays for childhood and adolescent neuropsychiatric disorders is ongoing. The condition currently under study is that of hyperactive children (HAC). Pharmacological compounds under study in these disorders include methylphenidate, amphetamine, piribedil, L-DOPA, tryptophan, Mianserin, clorgyline, and desipramine. Piribedil is safe but clinically ineffective in HAC while L-DOPA is minimally clinically effective. Tryptophan is effective on attention measures. Pharmacokinetic studies with clinical responses are included. Amphetamine half-life in children is about one-third that of adults. Behavior and motor activity responses to d-amphetamine occur during the absorption phase as determined by serial plasma amphetamine following a single dose. Central neurotransmitters and their metabolites are being studied in plasma and urine. Urinary 3-methoxy-4-hydroxyphenylglycol (MHPG) shows a time-related decrease during treatment with d-amphetamine; dopamine metabolites are unchanged. Tyramine and its metabolites are also decreased following d-amphetamine, whereas phenylethylamine is greatly increased following d-amphetamine.

Project Description:

Objectives: The purposes of this program are broad. An objective is to gain new knowledge of the central nervous system (CNS) of children and adolescents with special reference to maturational changes and neuropsychiatric disorders. Compared to the neurobiology known in adult neuropsychiatry, considerably less is known regarding the neuropsychiatric disorders of children. A particular focus of these studies has been the relationship between neurotransmitter change in hyperactive children (HAC) following compounds that have major actions on central neurotransmitter metabolism. The study of d-amphetamine (d-AMPH), a compound with clear and reliable effects in HAC, has been of particular interest, its pharmacokinetics, its effects on catecholamine and indoleamine metabolism and on behavior, and the interrelationships of these effects.

There have been a number of hypotheses relating catecholamine metabolism and hyperactivity in children. The possibility of an overly active catecholaminergic system was first advanced. Later, a functional deficiency in catecholamines in HAC was proposed with the greater focus on the possibility of a functional dopamine (DA) rather than norepinephrine (NE) deficiency. Other biochemical alterations, particularly involving serotonin (5-HT) have also been proposed. More recently, alterations in phenylethylamine (PEA) have also been proposed. No single neurotransmitter system to date can be shown to have an etiological role. Dr. Michael H. Ebert and Dr. Judith L. Rapoport have provided overall collaboration and support for this multi-faceted project.

Methods Employed: An inpatient and day patient program for children and adolescents, involving selected overnight stays, is ongoing on an inpatient nursing unit. Children who are hyperactive, aggressive and impulsive, and who have learning difficulties, have been admitted in order to study a carefully defined population of HAC. Children and adolescents with other conditions have also been studied. Specific exclusion and inclusion criteria are employed.

All children are thoroughly evaluated by medical, psychiatric, and psychometric examinations with all routine and other indicated procedures and clinical laboratory studies. Children also receive a psycholinguistic examination in collaboration with NINCDS. Neurological examinations are scored carefully according to a rating scale (PANESS). Several clinical and behavioral rating instruments have been utilized.

Pharmacological compounds, both standard and those previously unused in children, are being studied. Serial plasma pharmacokinetic data are being generated for d-AMPH. These data are studied in conjunction with motor activity, behavior, cognition, speech, temperature, and cardiovascular response. Piribedil, a specific DA agonist, and L-DOPA have been given to HAC. Mianserin, a NE agonist; tryptophan (TP), a precursor of 5HT; clorgyline, a monoamine oxidase inhibitor (MAOI); and desipramine, a tricyclic antidepressant; have all been administered to HAC in clinical trials. Fenfluramine trials are underway.

Motor activity is measured by an ambulatory activity monitor with solid state memory which measures individual motor movements via a pendulum acceleration system per unit of time and set at a desired sensitivity for the particular

study. At any time the instrument can be read into a computer for a print-out. Behavioral changes in HAC are measured via Conners' Teachers' Rating Scale (CTRS). Cognition is measured by a continuous performance task (CPT) in which errors of omission and commission can be scored in terms of differing time intervals. Time intervals can also be increased or decreased in relationship to accuracy of response.

d-AMPH is measured by radioimmunoassay (RIA) and gas chromatograph mass spectrometry (GC-MS). Biochemical studies include 24-hour urine collection to study NE, 3-methoxy-4-hydroxyphenylglycol (MHPG), vanillylmandelic acid (VMA), and normetanephrine (NMN); DA, homovanillic acid (HVA), 3,4-dihydroxyphenylacetic acid (DOPAC) and 3-methoxytyramine (3-MT); tyramine (TRM) and parahydroxyphenylacetic acid (PHPA); and phenylethylamine (PEA) and phenylacetic acid (PAA) in collaboration with Dr. Alan J. Zametkin. Plasma pharmacokinetics of pharmacological compounds are being ascertained. Plasma NE, MHPG, NMN, and VMA changes as they relate to plasma d-AMPH levels have also been studied. Neurophysiological studies include routine and sleep EEG's and EMI scans when indicated. Averaged evoked response (AER) studies are being conducted as they relate to HAC in drug-free and treated conditions. Psycholinguistic changes, in collaboration with Dr. Christy L. Ludlow of NINCDS, are also studied in relation to d-AMPH, piribedil, and TP effects. Paired associate learning has also been assessed in different drug conditions. Chronic effects of d-AMPH (2 weeks) are being studied with regard to pharmacokinetics and clinical response, particularly in terms of evidence for tolerance or supersensitivity and effect on neurotransmitter metabolism, as manifested by changes in plasma NE, MHPG, HVA, and dopamine-beta-hydroxylase (DBH) and platelet 5HT and MAO. The effects of TP and valine and d-AMPH and placebo are being measured with regard to behavior, attention, rectal temperature, motor activity, and plasma amino acids and indoleamines.

Major Past Findings: Serial plasma pharmacokinetic data indicate that d-AMPH reaches a peak level in children within 3-4 hours of an initial dose; however, as much as 70-80% will remain in the serum at 5-6 h when behavioral effects have largely dissipated. Mean apparent elimination half-life is 6.8 ± 0.5 h. Test-retest studies of individuals indicate that both pharmacokinetic data and clinical response data are highly replicable. Sustained release capsules produce a slower rate of absorption and a more plateau-like, longer lasting peak level, but do not give a prolonged clinical response. Socially appropriate behavioral change and motor activity decrease is maximal at 1-3 h after administration of a single dose (0.5 mg/kg) of d-AMPH. Clinical changes may be related to a release of catecholamines and the subsequent depletion of their stores, replacement by a "false" neurotransmitter metabolite of AMPH, or to alteration in receptor sensitivity. Higher single doses (1.0 mg/kg) effect earlier similar clinical responses, but of less magnitude. Piribedil is safe but clinically ineffective in HAC. In one study, d-AMPH has also been shown to have an anti-aggressive effect in those HAC with a considerable degree of conduct disorder. In another recent study, whose report is still in preparation, preliminary results indicate that neither TP nor valine (a neutral amino acid which competes with TP and inhibits its crossing the blood-brain barrier) results in behavioral response or basal temperature change after a single dose but attention span increase is similar to that observed following d-AMPH, while there are clear effects on plasma amino acids in the expected directions. This study was also designed such

that the effects of the procedure itself could be accounted for. On the other hand, d-AMPH after a single dose appears to have no effect on serial plasma amino acids, 5HT, or 5-hydroxyindoleacetic acid (5HIAA) over a 6 h period. This preliminary finding could be quite important in that d-AMPH has been shown to have clear effects on central 5HT in animals. Another recent study, also in preparation, indicates that both plasma MHPG and HVA are affected acutely by single-dose d-AMPH in a non-pretreated child, but this biochemical response may not be the same following two weeks of d-AMPH. Further analysis of this study may have implications for receptor change.

Urine studies indicate that day and night excretion of MHPG and HVA are not different; however, d-AMPH after 8 and 14 days is associated with lower MHPG levels. Behavioral response may be associated with the decrement in MHPG. Urinary HVA is unchanged. These biochemical and behavioral findings have been replicated in a subsequent HAC group, not yet published, as well as extended to other metabolites of both NE and DA. TRM and PHPA excretion are also decreased and PEA excretion is markedly elevated following two weeks of d-AMPH. PEA excretion is lower in HAC versus controls; its significance depends on whether it is expressed in terms of creatinine excretion. The TRM change may be associated with cardiovascular response and partially indicative of the change in PEA metabolism. More recent preliminary studies indicate a different pattern of metabolite response to methylphenidate (MP), a drug which produces a behavioral effect similar to d-AMPH. Though the effects are essentially opposite following MP with regard to NE and its metabolites, both d-AMPH and MP effect no change in DA or its metabolites.

HAC are not different from normals with regard to plasma NE and DBH but do have significantly more neurological soft signs by PANESS examination. New item analysis data indicates the prevalence of varied soft signs and their rater reliability. Plasma NE correlates with anxiety ratings and changes both with regard to dose of d-AMPH and time following dose, with higher doses of d-AMPH (1.0 mg/kg) giving strongest response at 1 hour and lower doses (0.5 mg/kg) giving strongest response at 3 hours. Elevated plasma NE is also associated with increases in blood pressure and pulse, and is dose-related. In a more recent study, baseline plasma NE, measured prior to an early a.m. dose of d-AMPH, does not change after two weeks of d-AMPH versus two weeks of placebo.

Preliminary results indicate a decreased platelet 5HT, no change in platelet MAO and an increase in plasma amine oxidase (AO) in HAC versus normals. Decreased platelet 5HT may be related to diet and platelet MAO correlates negatively and significantly with age.

With regard to pharmacological response, d-AMPH is effective and piribedil and L-DOPA are minimally so; TP produces responses similar to d-AMPH. HAC with higher levels of soft signs have more abnormal EEG's, more minor physical anomalies, lower full-scale I.Q.'s (WISC-R), and a greater number of errors on the Bender. Data from psycholinguistic evaluations indicates that HAC have impairments in certain auditory processing and language skills; furthermore, d-AMPH does not evoke pronounced effects with regard to language performance in HAC vs. normals; older and less hyperactive subjects showed the most improvement. Improvement in cognitive parameters was shown only in normals.

New Findings: No new data has been collected by Dr. Brown, though several of the more recent findings above are in preparation for publication.

Significance to Mental Health Research: Though childhood neuropsychiatric disorders, and particularly HAC, have been considerably studied in the last few years, there are many diagnostic, psychopharmacological and psychobiological questions yet to be answered. Many studies in the past in child psychiatry have been related to psychological, psychodynamic issues. As regards HAC, obsessive-compulsive children, enuretics, Gilles de la Tourette's syndrome, anorexia nervosa, psychoses, and autism, an increased interest in psychopharmacology has emerged. Though methylphenidate and AMPH give positive responses in 80% of well diagnosed HAC, the pharmacokinetics and metabolism of these drugs have been studied only relatively recently. One avenue to ascertaining possible neuropathology in these conditions is to understand more clearly the mechanisms of action of those pharmacological compounds which effectively alter the clinical conditions under study. The relationship between such basic pharmacological knowledge and clinical effects has been under-studied in children in general, though the work of this group has greatly enhanced the knowledge of neuropharmacology and neurotransmitters in childhood mental illness. More importantly, for the future, basic biological factors in childhood neuropsychiatry which might elucidate the psychopharmacological responses are, at this point, only hypotheses. The degree to which these hypotheses are validated or refuted could play a significant role in our understanding of childhood neuropsychiatry.

Proposed Course of Project: The principal investigator, Dr. Brown, remains in the Office of the Chief, BPB, but is no longer administratively a part of the Child Psychiatry Branch. The last active subjects from the present general project were completed during the spring of 1983. There is a considerable body of data yet to be analyzed but some of this is in preparation and in press and will be reported in the future. Dr. Robert M. Post, Chief, BPB, provides collaboration and support.

Publications:

Langer, D.H., Fletcher, J.C., Brown, G.L., Nee, L.E., and Smith, M.A.: Ethical considerations in psychological research in children. In Greenhill, L. and Shopsin, B. (Eds.): The Psychobiology of Childhood: Profiles of Current Issues. New York, Spectrum Publications, pp. 19-30, 1984.

Brown, G.L., and Ebert, M.H.: Catecholamine metabolism and hyperactive children. In Lake, C.R. and Ziegler, M.G. (Eds.): The Catecholamines in Psychiatric and Neurological Disorders. Stoneham, Maine, Butterworth Publishers, in press.

Ludlow, C., Cudshy, E., Bassich, C., and Brown, G.L.: Auditory processing skills of hyperactive, language-impaired and reading disabled boys. In Katz, J. and Lasky, E.Z. (Eds.): Central Auditory Processing Disorders: Problems of Speech, Language, and Learning. Baltimore, University Park Press, pp. 163-184, 1984.

Brown, G.L., Ebert, M.H., Minichiello, M.D.: Biochemical and pharmacological aspects of attention deficit disorder. In Bloomingdale, L.M. (Ed.): Attention Deficit Disorder. New York, Spectrum Publications, in press.

Amery, B., Minichiello, M.D., Brown, G.L.: Aggression in hyperactive boys: Response to d-amphetamine. J. Am. Acad. Child Psychiatry, 23, 3:291-294, 1984.

Zametkin, A.J., Karoum, F., Rapoport, J.L., Brown, G.L., Wyatt, R.J.: Phenylethylamine excretion in attention deficit disorder. J. Am. Acad. Child Psychiatry, 23, 3:310-314, 1984.

Zametkin, A.J., Brown, G.L., Karoum, F., Rapoport, J.L., Chuang, L.W., Langer, D.H., Wyatt, R.J.: Urinary phenylethylamine response to d-amphetamine in boys with attention deficit disorder. Am. J. Psychiatry, in press.

Zametkin, A.J., Karoum, F., Rapoport, J.L., Brown, G.L., Chuang, L.W., Wyatt, R.J.: Stimulant, urinary catecholamines and indoleamines in hyperactivity: a comparison of methylphenidate and dextrosamphetamine. Arch. Gen. Psychiatry, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00070-11 BP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Psychological and Biological Interactions in the Mood and Anxiety Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Robert M. Post, M.D. Chief BP NIMH

COOPERATING UNITS (if any) NSB, APB, CNB, CPB, LCM, LCS, LPP, RSB, IRP, NIMH; DEB, NICHD; DPCBR, NIAAA; PDS, NIH; USUHS, Dept of Def.; Univs. of CA, Chicago, VA; VA Med. Center Bronx; VA Med. Center, San Diego; Tufts Univ.; Univ. Hosp., Munich; Wellesley Hosp., Toronto; Thos. Jefferson Univ., Walter Reed Med. Cntr

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Section on Psychobiology

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, MD 20205

TOTAL MAN-YEARS:

12.0

PROFESSIONAL:

6.0

OTHER:

6.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Patients suffering from manic, depressive, schizoaffective, and anxiety-related disorders are longitudinally evaluated and treated. Double-blind, placebo-controlled clinical trials are employed. Classical neurotransmitters and their metabolites, as well as hormones and peptides implicated in the regulation of mood and behavior, are measured in blood and CSF of patients to assess their relationship to normal and pathological behavior. Alterations in cognitive function, neurophysiology, and biochemistry are explored in relationship to predictors and mechanisms underlying clinical response to treatments of mood and anxiety disorders with agents including anticonvulsants, noradrenergic receptor agonists, sleep deprivation, lithium carbonate, and other antidepressant modalities. Ongoing clinical trials with carbamazepine indicate it is a new and useful alternative to lithium carbonate for the acute and prophylactic treatment of manic-depressive illness. The biochemical and physiological effects of carbamazepine are being explored in the hope they will not only elucidate its mechanisms of action in affective illness, but provide a basis for better understanding the processes underlying affective dysregulation. Animal models of electrical kindling and stimulant-induced behavioral sensitization are explored in order to examine mechanisms underlying progressive changes in behavioral pathology.

COLLABORATORS:

Dr. T. Colburn, Research Services Branch, NIMH
Dr. W. Mendelson, Clinical Psychobiology Branch, NIMH
W. Duncan, Clinical Psychobiology Branch, NIMH
Dr. H. Holcomb, Biological Psychiatry Branch, NIMH
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Dr. T.W. Uhde, Biological Psychiatry Branch, NIMH
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Dr. G. Chrousos, Senior Investigator, Developmental Endocrinology Br., NICHD

I. Project Description

A. Objectives

This project is engaged in the multidisciplinary longitudinal study and treatment of patients with a spectrum of acute and chronic psychoses, particularly involving mood and anxiety disorders. Both investigative and treatment approaches focus on the elucidation of psychological and biological phenomena and their complex interaction.

B. Methods Employed

1. Subjects

a. Subjects who meet Research Diagnostic Criteria (RDC) for manic-depressive or schizoaffective illness or the more recent DSM III criteria for a spectrum of mood disorders are admitted to the 3-West Clinical Research Unit, Section on Psychobiology. Patients with anxiety and panic anxiety are also admitted to the unit under other protocols (see Project Z01 MH 00071-04 BP).

b. Normal volunteers are also admitted to the unit to provide control data for specific studies in patients and to assess clinical and biological interrelationships in normal as well as patient populations. Volunteers complete an extensive battery of biochemical, psychological, and physiological tests including lumbar punctures (LP's). LP's are performed at 9 AM and 9 PM to study alterations in circadian rhythms in patients and volunteers.

2. Psychological and Biological Evaluation

a. Behavior and Cognition: During an initial drug-free interval patients undergo extensive neurological, psychological, biochemical, and neurophysiological evaluation, including EEG-monitored sleep, averaged evoked potentials, and a variety of cognitive tests. These include the Halstead Category Test, a psychosensory questionnaire, a neuropsychological profile using the Luria Test, and an extensive battery of tests developed in collaboration with K. Squillace and A.F. Mirsky of the Laboratory of Psychology and Psychopathology.

Longitudinal behavioral data are collected in a double-blind fashion utilizing twice-daily global ratings by trained nursing observers. Patients also complete twice-daily ratings of mood and side effects in order to examine diurnal variation. Using the same double-blind methodology, nurses also evaluate patients on a modified Brief Psychiatric Rating Scale (BPRS) three times weekly.

b. Life Chart Methodology: A life chart technique has been developed to plot the number and severity of affective episodes and the interval between episodes so that the longitudinal development, recurrence, and progression of the illness can be accurately quantitated and illustrated. This technique is an important clinical as well as research tool for assessing the efficacy of treatment interventions.

c. Physiology: Motor activity is measured continuously at 15-minute intervals with a miniaturized activity monitor developed by Dr. T. Colburn. EEG-monitored sleep is studied in collaboration with Dr. W. Mendelson and W. Duncan. In collaboration with Drs. Richard Coppola, Henry Holcomb, and M.S. Buchsbaum, 16-channel EEG's, averaged evoked responses, and studies of hemispherical laterality and psychophysiological pain are conducted.

- d. Functional Anatomy: In addition to computerized axial tomography (CAT-scan) evaluation of our patients for possible cerebral pathology, studies have been initiated in collaboration with Drs. Robert Cohen, Lynn DeLisi and M.S. Buchsbaum and associates to study regional functional activity of the brain using (18F) flurodeoxyglucose.
- e. An alpha-Adrenergic Agonist, Clonidine: Clonidine is administered intravenously to depressed and anxious patients and volunteers in order to assess clinical, physiological, and neuroendocrine responses to this alpha-adrenergic agonist (with Drs. T.W. Uhde, L. Siever and D.L. Murphy).
- f. alpha-Adrenergic Receptors: In collaboration with Dr. M. Kafka, platelet alpha receptor function, as well as prostaglandin-stimulated increases in cyclic-AMP and their inhibition by norepinephrine, are assessed in normal volunteers and patients with mood and anxiety disorders.
- g. Urinary Free and Plasma Cortisol; Dexamethasone Suppression; CRF Stimulation; and Lymphocyte Glucocorticoid Binding: Basal 24-hour urinary free cortisol is measured during depressed and manic states in medication-free conditions and during treatment. A detailed evaluation of the pituitary-adrenal axis is conducted by Dr. D.R. Rubinow in patients with affective illness and anxiety disorders. Plasma cortisol is measured under basal conditions and following the dexamethasone suppression test. Glucocorticoid receptor binding on lymphocytes is assessed in collaboration with Dr. D.C. Jimerson. Hormonal response to CRF before and during treatment with carbamazepine is studied with Dr. P.W. Gold (see below).
- h. Cerebrospinal Fluid (CSF) and Plasma Studies: Plasma and CSF studies comprise an important area of biological evaluation of classical neurotransmitters and their amines, as well as the newly discovered peptide substances, in normal volunteers and in patients during ill and well intervals. These studies are conducted in collaboration with Drs. D.R. Rubinow, P.W. Gold, D.C. Jimerson, and M. Linnoila, as well as many investigators within and outside of NIMH, with specialized techniques for measurement of specific peptide hormones. Several measures of GABA metabolism are obtained in plasma or CSF of affectively ill patients or controls in collaboration with Drs. W. Berrettini and T. Hare.
- i. Oxytocin and Vasopressin: In collaboration with Drs. H. Weingartner, P.W. Gold, and D.R. Rubinow, infusions of these peptides are utilized to assess effects on memory, mood, and endocrine function in affectively ill patients and normal volunteers.
- j. Adrenergic Mechanisms of Cortisol Hypersecretion: Propranolol and thymoxamine are administered to affectively ill patients and controls to assess whether cortisol hypersecretion is under the regulatory control of the adrenergic system (in collaboration with Drs. L. Bierer and D. Jimerson).
- k. Corticotropic Releasing Hormone: CEF is studied in collaboration with Dr. P.W. Gold.

1. Procaine Activation: Procaine, an agent which activates limbic system structures with some selectivity, is administered intravenously in graded doses to affectively ill, borderline, and normal subjects to assess possible altered behavioral, electrophysiological, or biochemical responsivity in this system. Collaborators include Drs. C. Kellner, B. Vittone, F. Putnam, R. Coppola, D. Gardner, and R. Cowdry.

m. Platelet Vasopressin Receptors: AVP receptors on platelets are studied in collaboration with Dr. W. Berrettini.

3. Treatment

a. Psychotherapeutic: Treatment and evaluation are conducted in individual and group therapy, and ongoing clinical case conferences are utilized.

b. Routine Somatic Treatment: Both routine and experimental compounds are evaluated during double-blind clinical trials. The routinely used drugs include tricyclic antidepressants, lithium carbonate, monoamine oxidase inhibitors, and neuroleptics. These agents are utilized not only because of their clinical efficacy, but as well to further understand their mechanisms of action and possible interaction with the pathophysiology of the illness.

c. Experimental Compounds: The anticonvulsant carbamazepine has been introduced as a new treatment for manic and depressive illness and is evaluated for its acute and prophylactic efficacy. Diphenylhydantoin and valproic acid are two other anticonvulsant agents also being studied in selected patients to assess the specificity of the positive psychotropic effects of carbamazepine in relation to other anticonvulsants with different spectrums of clinical efficacy.

d. Receptor Agonists: Clonidine, in addition to acute intravenous studies, is administered during clinical trials to assess the clinical efficacy of alterations in adrenergic functioning in anxiety and affective illness.

e. Peptide Strategies: In addition to acute challenges with oxytocin and vasopressin, clinical trials have been conducted in collaboration with Dr. P.W. Gold of a vasopressin analog, DDAVP, in affective illness.

f. Sleep Deprivation: The paradoxical antidepressant effects of one night's sleep deprivation in depressed patients are explored both to develop a model for further understanding the rapid onset and offset of a non-pharmacologically-induced mood improvement and to assess its therapeutic potential.

4. Animal Models.

A rodent behavioral pharmacology laboratory is maintained in collaboration with Drs. S. Weiss and A. Pert to develop new research techniques in several areas. The longitudinal evolution of behavioral pathology is assessed using a number of paradigms including: 1) electrophysiological kindling; 2) pharmacological kindling; 3) behavioral sensitization to psychomotor stimulants and related dopaminergic agonist compounds; and 4) the evaluation of stress

sensitization and learned helplessness and their possible underlying neural substrates. Physiological and biochemical changes, particularly alterations in receptor binding, are studied in collaboration with Drs. A. Pert, P. Marangos, J. Patel, and D. Jacobowitz. ¹⁴C-2-deoxyglucose studies have been conducted utilizing pharmacological kindling with lidocaine in collaboration with Drs. C. Kennedy, L. Sokoloff, and associates. The role of seizures in the development of behavioral pathology is studied utilizing a variety of seizure models, behavioral assessments, and anticonvulsant compounds.

C. Major Findings

1. Carbamazepine: A New Treatment for Manic-Depressive Illness

Last year, Dr. Post received the Foundation's Fund Prize Award for Research in Psychiatry given by the American Psychiatric Association for his work on carbamazepine and its theoretical underpinnings.

a. Introduction: Several empirical observations and theoretical perspectives led to our initiation of the first double-blind, placebo-controlled clinical trials of carbamazepine in mania and depression in the U.S. There had been persistent reports of positive effects on mood and behavior in epileptic patients treated with carbamazepine. Carbamazepine, both clinically and in experimental models such as kindling, is the most effective anticonvulsant against temporal lobe-limbic seizures. Temporal lobe and limbic structures have long been hypothesized to be importantly involved in the modulation of normal and pathological affect. As such, an agent which stabilized abnormal excitability in this area of brain might be expected to have stabilizing effects on emotional function. Moreover, preliminary data from open clinical trials in Japan suggested that carbamazepine might be effective in manic-depressive patients when it was added to previously ineffective drug regimens.

b. Acute Antimanic Efficacy: In collaboration with Dr. T. Uhde, we continue to document unequivocal evidence of the efficacy of carbamazepine in the acute treatment of manic episodes. It is noteworthy that this occurs in many patients who were previously nonresponsive to lithium carbonate, the more traditional agent for the treatment of affective illness. Evidence of carbamazepine response has been documented during an "off-on-off-on" design where carbamazepine and placebo are administered in an alternate fashion, with nurses blind to this clinical trial. We have noted repeated clinical improvement during carbamazepine treatment and exacerbation during placebo substitution. The time course of improvement parallels that of neuroleptics.

c. Acute Antidepressant Efficacy: Twenty-two of the first 37 patients have shown evidence of clinical response to carbamazepine. In some instances, marked clinical improvement was observed in previous drug nonresponders but in most the improvement was mild to moderate. Relapses following placebo substitution were not as consistently observed in the depressive phase as they were in the use of carbamazepine for the treatment of mania. Therefore, like lithium, carbamazepine appears to have clear antimanic but less well-documented acute antidepressant properties. Degree of antidepressant response correlated with CSF and plasma levels of carbamazepine-10,11-epoxide. Patients with more severe depression responded better to carbamazepine than those with less severe

initial ratings of depression on placebo. Family history of affective illness did not predict response, although acute antidepressant response to sleep deprivation did predict subsequent response to carbamazepine. Those with more rapid cycling (episodes/years ill) and hospitalizations for mania, but fewer total weeks depressed (i.e., less chronic depression), also responded better. In responders, motor activity increased in the evening hours, but baseline activity profiles, studied by Dr. R. Joffe, did not predict carbamazepine response.

d. Prophylactic Efficacy of Carbamazepine: Nine patients have been followed for a mean of 2.7 years on carbamazepine in either a double-blind or an open fashion. In these lithium-nonresponsive, rapidly cycling manic-depressive patients, carbamazepine decreased the mean number of affective episodes per year from 17.0 in the years prior to carbamazepine treatment to 6.5 episodes/year on the drug. The severity and duration of episodes when they did occur were also reduced on carbamazepine. Discontinuation of the drug resulted in relapses in five of six patients, further indicating that improvement was related to carbamazepine and not to spontaneous improvement in the course of illness.

e. Side Effects: The drug is well tolerated in the majority of patients, with mild and clinically insignificant decreases in white count observed in the majority of patients. No patient had to be dropped from the trial because of a low white count. Rashes were observed in 10-15% of patients requiring drug discontinuation. Mild decreases in serum sodium and calcium are also observed, as documented by Drs. R. Joffe and T. Uhde. Sedation and dizziness are dose-related and tend not to occur with slow increases in dose. Analysis of self-ratings of side effects indicates that depressed patients experience a moderate incidence of apparent "drug-related" side effects while medication-free on placebo, and while on carbamazepine show a similar profile of "side effects". The effects of carbamazepine on thyroid function elucidated by Drs. P. Roy-Byrne and R. Joffe have led to a major reconsideration of the role of thyroid hormones in affective illness (see section h,2 below).

While daytime sedation is not a problem, substantial improvement in sleep has been noted in half-hour sleep checks by nurses blind to active carbamazepine administration. In the first 27 depressed patients, sleep significantly increased ($p < .001$) during the first week of carbamazepine. This often preceded clinical improvement in depressed mood and was maintained during the clinical trial. Similarly, in the first 11 manic patients studied, sleep almost doubled in the first week of carbamazepine administration ($p < .001$). EEG studies in collaboration with Drs. W. Mendelson and J.C. Gillin and W. Duncan will document the stages of EEG-monitored sleep that are affected.

f. Plasma and CSF Levels of Carbamazepine and its -10,11-Epoxy Metabolite: Spinal fluid levels of carbamazepine and its 10,11-epoxy metabolite were measured in 18 affectively ill patients. These studies were performed in collaboration with Drs. T.W. Uhde, D.C. Chatterji, and R.F. Greene. Mean CSF carbamazepine was 2.06 ± 0.10 ug/ml, while the epoxy was $0.91 \pm .09$ ug/ml or 44% of the concentration of the parent compound. Carbamazepine levels in plasma or in CSF (a measure of free carbamazepine) were not significantly related to degree of clinical antidepressant or antimanic response.

However, CSF levels of carbamazepine-10,11-epoxide were significantly correlated with the degree of clinical response ($r = .67$, $p = .005$). Similar relationships were also observed in plasma where the epoxide, but not carbamazepine itself, was correlated with the degree of clinical response. These data suggest that in those patients treated with an average of 1000 mg/day of carbamazepine, achieving plasma levels between 6 and 12 ug/ml, there is not a close relationship between carbamazepine levels and clinical response. Similar observations have been made in the neurological literature in relationship to anticonvulsant efficacy. However, our data suggest the possibility that the 10,11-epoxide metabolite, which we and others have noted to have anticonvulsant effects in animals, may also possess active psychotropic properties in man.

g. Comparison with Other Anticonvulsants: Clinical trials have been initiated to examine the relative efficacy of carbamazepine in comparison to other anticonvulsants such as phenytoin and valproic acid. In the first patient to complete a double-blind crossover design, no evidence of clinical improvement was observed with phenytoin or valproic acid, while the patient was an unequivocal carbamazepine responder. These data suggest the possibility that biochemical or physiological properties peculiar to carbamazepine may, at least in this patient, be important to its psychotropic properties rather than relating to generalized anticonvulsant effects. Emrich and associates in Europe, and investigators in five other countries, have, however, reported the successful use of valproic acid in a small number of lithium-resistant manic-depressive patients. Further clinical trials of these agents are indicated.

Although carbamazepine is a highly effective anticonvulsant, it is also useful in the treatment of a variety of paroxysmal pain syndromes which clearly do not involve an ictal process. Thus, the efficacy of carbamazepine does not imply that subclinical seizures are the underlying pathophysiological mechanism in patients with affective illness. However, the properties mediating carbamazepine's anticonvulsant effects may nonetheless be related to its psychotropic properties. The clinical utility of the anticonvulsant carbamazepine raises the paradox of why the major motor seizures of electroconvulsive therapy are among the most effective treatments for acute manic and depressive illness. As detailed below, we have documented that electroconvulsive seizures in the rat are paradoxically anticonvulsant to amygdala-kindled seizures. These data raise the possibility that common biochemical and physiological mechanisms of electroconvulsive therapy in man and the anticonvulsant carbamazepine on limbic system excitability could be related to their profile of therapeutic efficacy in both phases of affective illness.

h. Studies of Carbamazepine's Mechanism of Action:

1) Effects on Classical Neurotransmitters and Modulators:

Evidence in laboratory animals (Purdy et al.) suggests that carbamazepine blocks the reuptake of norepinephrine (NE) but also inhibits stimulated-induced release. We have observed, in collaboration with Dr. D.C. Jimerson and E. Gordon, that carbamazepine treatment significantly reduces the NE metabolite 3-methoxy-4-hydroxyphenylethylene glycol (MHPG) in CSF of patients with affective illness. CSF NE itself, measured in collaboration with Drs. C.R. Lake and M. Linnoila, is not

significantly altered in the depressed patients; however, the elevated levels of CSF NE in mania are decreased by carbamazepine. Noradrenergic effects of carbamazepine have indirectly been linked to its anticonvulsant properties. CSF 5-hydroxyindoleacetic acid (5HIAA) is also not significantly affected by carbamazepine.

The possible dopaminergic effects of carbamazepine are of considerable interest, but presently remain to be further clarified. There is substantial indirect evidence that carbamazepine does not act as a classical neuroleptic. It does not appear to block cocaine- or amphetamine-induced hyperactivity or stereotypy and does not raise HVA levels in rat brain or in the spinal fluid of our patients with affective illness, as do the classical neuroleptic treatments. Moreover, it has not been associated with the development of parkinsonian side effects or with the syndrome of tardive dyskinesia as have the neuroleptic drugs. Interestingly, carbamazepine produces slight but statistically significant increases in serum prolactin in contrast to the major increases in prolactin achieved by traditional antipsychotic agents. It does not displace ³H-spiroperidol binding (Marangos et al, 1983) or increase firing of dopamine neurons in the substantia nigra (P. Clark and D. Hommer). These data indicate that carbamazepine has differential effects on dopaminergic mechanisms that could be important to its profile of effects and side effects in epilepsy and affective illness.

Alterations in GABA have been postulated in affective illness (see below) as well as in the seizure disorders. Carbamazepine has been reported to decrease the turnover of GABA in animal studies (Bernasconi, 1984), although brain levels are not altered by the drug. This is consistent with our data indicating that CSF GABA levels are not significantly decreased during treatment with carbamazepine compared to baseline levels.

Effects of carbamazepine on central and "peripheral" benzodiazepine receptors have been studied with biochemical techniques (Marangos et al.) and electrophysiologically in the amygdala kindling model. Carbamazepine binds poorly to the central site (³H-diazepam or ³H-BCCE), but more potently at the Ro5-4864 (peripheral) site. In parallel, Dr. S.R.B. Weiss has found that Ro-15-1788 blocks the anticonvulsant actions of diazepam, but is ineffective in reversing the anticonvulsant effects of carbamazepine on amygdala kindling. Conversely, Ro5-4864 does reverse carbamazepine's anticonvulsant effects, but not those of diazepam. Taken together, these biochemical and electrophysiological data suggest the possibility that carbamazepine may have physiologically relevant effects on the Ro5-4864 site in brain. PK11195, an antagonist at the peripheral site, blocks the effects of Ro5-4864 against carbamazepine, further supporting an effect through this mechanism.

Carbamazepine is potent in displacing binding of several adenosine receptor ligands (Marangos et al.). However, the anticonvulsant efficacy of 12 carbamazepine analogs on ECS seizures in mice does not correlate with the ability to displace either adenosine agonist or antagonist binding. Several adenosine agonists and antagonists also do not affect carbamazepine's effects on amygdala kindling. These data suggest that carbamazepine's potent effects on amygdala kindling may be related to a property of the drug other than its adenosine effects.

Cyclic nucleotides (cyclic-AMP and cyclic-GMP) have been postulated to play an important role in the therapeutic effects of a variety of psychotropic and anticonvulsant agents. While carbamazepine did not affect basal levels of these in the CSF of our affectively ill patients, probenecid-induced accumulations tended to be significantly reduced.

2) Carbamazepine's Effects on Endocrine and Peptide Systems:

Carbamazepine significantly decreased somatostatin measured in CSF of affectively ill patients in collaboration with Drs. D.R. Rubinow, P.W. Gold, and S. Reichlin. These findings are of interest in relationship to the report of long-lasting increases in brain somatostatin following amygdala kindling seizures and the findings of low CSF somatostatin in depressed patients. These findings could relate to the anticonvulsant properties of carbamazepine as others have recently demonstrated that blocking somatostatin function inhibits seizures.

Carbamazepine's effects on vasopressin are noteworthy from both a clinical and theoretical perspective. In contrast to lithium carbonate, which produces the diabetes insipidus syndrome, carbamazepine has been used to treat diabetes insipidus. It has antidiuretic properties which are manifest by its effects in producing mild hyponatremia (Drs. R. Joffe and T.W. Uhde). During carbamazepine treatment, decreased endogenous vasopressin is secreted in response to a hypertonic saline load, also consistent with an agonist role in this system (Drs. P.W. Gold and J.C. Ballenger). These findings are opposite those observed during lithium carbonate treatment. Dr. W.H. Berrettini has documented that carbamazepine is the one psychotropic drug tested to date that displaces ¹²⁵I-arginine vasopressin binding from platelets, further suggesting that carbamazepine may have direct effects at the vasopressin receptor. The relationship of carbamazepine's anti-diuretic effects to possible alterations in mood and cognition remain to be explored.

The effects of carbamazepine on cortisol are noteworthy from several perspectives. Rubinow and associates have found that carbamazepine induces escape from dexamethasone suppression, even in depressed patients who are showing clinical improvement. It is unlikely that the effects of carbamazepine on dexamethasone metabolism entirely account for escape from dexamethasone suppression, as urinary free cortisol is also increased by carbamazepine in patients with initially normal levels. Carbamazepine may thus be affecting regulation of the pituitary-adrenal axis directly, or through its effects on higher neural substrates in the limbic system or elsewhere. The carbamazepine-induced decrease in somatostatin described above could account for the effects on cortisol, as somatostatin has been shown by others to inhibit CRF stimulation of ACTH. The vasopressin agonist properties of carbamazepine could also account for the cortisol effects, as vasopressin appears to stimulate cortisol secretion in man (Rubinow et al.).

The possible effects of carbamazepine on endogenous opiate systems are of interest in relation to the efficacy of carbamazepine in pain syndromes and the fact that it potentiates opiate-induced running activity in mice. There was no significant effect of carbamazepine on CSF opiate binding activity in 17

affectively ill patients, studied in collaboration with Drs. D. Naber, D. Pickar and associates. Discrete effects of carbamazepine on regional opioid sub-systems in brain remain to be ruled out, however.

In contrast to lithium, carbamazepine inhibits rather than potentiates the TSH response to TRH (Drs. R. Joffe and P.W. Gold). Like lithium, carbamazepine decreases plasma levels of T_3 , T_4 , and free T_4 in a highly significant fashion, but it does not result in a marked increase in basal TSH or in clinical hypothyroids, as may occur with lithium. Drs. P. Roy-Byrne and R. Joffe have found, paradoxically, that those patients with the greatest decrease in T_4 and free T_4 were the best responders to carbamazepine. This has led Dr. Joffe to a new conceptualization of the role of thyroid hormones in affective illness (see below).

Continued study of carbamazepine's biochemical effects, either alone or in comparison and contrast to lithium carbonate, may ultimately prove useful not only in further understanding its mechanism of action in affective illness, but also in helping to understand substrates underlying the affective disorders.

2. Approaches to Neurotransmitter Receptor Dysfunction in Affective Illness

a. Noradrenergic Systems: alpha-Adrenergic receptors have been measured on platelets of drug-free patients with affective disorders and normal control subjects in collaboration with Dr. M. Kafka. The number of receptors measured by 3H -dihydroergocryptine was significantly increased in patients, while noradrenergic inhibition of prostaglandin PGE_1 -stimulated cyclic-AMP production was significantly reduced. Parallel findings have been observed in panic-anxious patients (Uhde et al.). In contrast to these measurements in platelets, endocrine responses have been blunted following the acute intravenous administration of the alpha-2 receptor agonist clonidine, in collaboration with Drs. T.W. Uhde, L.J. Siever, and D.L. Murphy. Last year, Drs. Uhde and Siever received the A.E. Bennett Prize for clinical research from the Society for Biological Psychiatry for this work and that described below.

Consistent with its effects on decreasing firing of the noradrenergic locus coeruleus in animals, clonidine acutely decreased plasma NE and MHPG, measured in collaboration with Drs. C.R. Lake and D.C. Jimerson. Clonidine was associated with antianxiety effects measured on the Spielberger Rating Scale in depressed and anxious patients. No significant effects on anxiety were observed following placebo administration or in the normal volunteer subjects. Clonidine's effects are consistent with the observations that CSF NE (Dr. R. Lake) and CSF MHPG (Dr. D.C. Jimerson) may be slightly elevated in some depressed patients (possibly those with greater anxiety) compared to normal volunteers. However, CSF NE is markedly increased in manic patients compared to either of the other patient or control populations. In normal volunteers plasma MHPG, measured by Dr. Jimerson, was observed to correlate significantly and negatively with severity of depression, hypochondriasis, and psychasthenia scales measured on the Minnesota Multiphasic Personality Inventory (MMPI). The data raise the possibility that noradrenergic mechanisms may be associated with the normal as well as pathological range of affective function. Measurements of noradrenergic function in blood, urine, and spinal fluid of these affectively ill patients, in collaboration with Drs. D.C.

Jimerson and J.C. Ballenger, are also helpful in clarifying the role of interrelationships between noradrenergic measures in different body fluids.

In addition to the state-related alterations in noradrenergic function, we have been interested in assessing the relationship of this system to the longitudinal course of affective illness, as assessed by life chart methodology. We have observed that those patients with higher CSF NE had greater numbers of episodes in the year prior to NIMH admission ($r = .61, p .05$). Those with higher CSF NE during the depressive state experienced greater numbers of weeks ill in the year prior to NIMH admission ($r = .76, p .001$). We have also followed a group of patients an average of 3.5 years to assess social functioning following discharge from NIMH (unpublished data with R.J. Savard). Patients who had poor social functioning measured in the social and leisure activity subscale of the Social Adjustment Scale had higher CSF VMA ($r = .66, p .02, n = 13$) and higher CSF NE ($r = .80, p .005, n = 11$). These findings, taken together, suggest that increases in noradrenergic function measured during an acute episode of depression may be positively related to the longitudinal course of affective illness and to greater frequency of cycling as well as poorer prognosis variables. These are among the first observations of biological correlates associated with the longitudinal, rather than acute state-related, course of affective illness.

b. GABA: In collaboration with Drs. W.H. Berrettini and R. Joffe, we have recently reviewed the literature on possible alterations in GABA-ergic mechanisms in affective illness. Indirect pharmacological data support a possible role of GABA in affective illness. Moreover, direct measurements of GABA in plasma and CSF provide some evidence of disturbed GABA function. Plasma GABA, measured in collaboration with Dr. T. Hare, was significantly lower in euthymic medication-free patients compared to normal controls. Four of five studies in the literature have reported low CSF GABA in depression compared to control groups. We have observed significantly lower levels in an individual studied longitudinally during depressed compared to manic phases of the illness. Dr. Berrettini, in conjunction with Dr. E. Gershon, has collected further evidence that GABA may, in part, be regulated at a genetic level as well as fluctuating in a state-related fashion. Plasma GABA levels were significantly correlated in identical twin pairs. Dr. Berrettini has also measured GABA transaminase (GABA-T) and found this enzyme to be significantly lower in affectively ill patients compared to normal volunteers. These studies, suggesting possible GABA alterations in affective illness, are of interest in relation to recent reports that GABA agonists may have antidepressant effects, and that several agents reported to be effective in the treatment of recurrent affective illness (electroconvulsive therapy, lithium, carbamazepine, and valproic acid) all decrease GABA turnover.

c. Dopamine: Indirect biochemical, pharmacological, and endocrine data continue to suggest a role for dopamine in some aspects of affective illness. Dopamine and its metabolite HVA and DOPAC are studied in collaboration with Dr. M. Linnoila in depressed, manic, and euthymic patients and controls. The relationship of plasma HVA (studied by Dr. R. Joffe) to the longitudinal course of affective illness is being assessed.

d. Serotonin: Dr. G.L. Brown has found that low 5HIAA in CSF of several patient populations is correlated with aggressivity directed externally or internally (suicidality). Dr. Roy-Byrne has found that this relationship is not observed in bipolar depressed patients, however. In hyperactive children, Dr. Brown has found that the serotonin precursor tryptophan alters amino acid interrelationships and has clinical effects similar to that of amphetamine. Studies of 5HIAA in relation to aggression, suicidality, and the longitudinal course of affective illness are being pursued further.

3. Thyroid Function in Affective Illness:

Based on the findings (mentioned above) that responders to carbamazepine showed greater decreases in T_4 and free T_4 than non-responders, R. Joffe examined other pharmacological data suggesting T_4 similar relationships. He found that responders to lithium had greater increases in TSH. ECT was also associated with decreases in circulating thyroid hormones (Joffe, unpublished data and Kirkegaard et al, 1975). Thus, three different treatments effective in mania and depression (carbamazepine, lithium, and ECT) all paradoxically appeared to alter peripheral indices of thyroid function in the direction of relative hypofunction. How might this be compatible with consistent and growing evidence that T_3 potentiates that antidepressant response to tricyclics? Dr. Joffe noted that T_3 treatment, by decreasing circulating T_4 , may also be inducing relative CNS thyroid hypofunction. In contrast to the periphery which is able to directly use T_3 , 80% of the brain's T_3 is derived from T_4 . Thus, T_3 potentiation may be a misnomer and may be acting by decreasing rather than increasing thyroid indices (especially T_4).

4. Peptides in CSF: Interrelationships with Neurotransmitter and Behavioral Alterations

a. Introduction: More than 20 neuropeptide substances have been suggested as putative CNS neurotransmitters or modulators. We have recently reviewed the literature indicating that essentially all of these substances have been tentatively identified and measured in the CSF of man. In many instances there is substantial evidence that CSF levels are regulated independently of those in the periphery and may be more closely associated with changes in brain. This provides one strategy for attempting to identify peptidergic alterations in neuropsychiatric disorders and to examine their postulated relationship to alterations in behavior, cognition, and affect. Neuropeptides have recently been reported to co-exist in the same neurons with classical neurotransmitter substances. Again, the CSF provides an opportunity for studying the potential interaction between both classical neurotransmitters and the recently discovered neuropeptides.

b. CSF Opiate-like Substances: In collaboration with Drs. D. Pickar, J.C. Ballenger, D. Naber, D.R. Rubinow, W.E. Bunney, Jr., and F.K. Goodwin, we have measured opiate-like substances in CSF utilizing a measure of both total CSF opiate binding activity and immunoreactive beta-endorphin. Total CSF opiate binding activity was not significantly different in depressed, manic or improved patients compared to normal volunteers. In depressed patients, those with higher nurse-rated anxiety showed significantly higher opiate binding activity ($r = .47$, $p = .01$, $n = 36$). Utilizing a different measure of anxiety,

i.e., self-rated state anxiety at the time of the LP, it was observed that normal volunteers with higher CSF opiate binding activity showed significantly lower levels of subjective self-rated anxiety ($r = -.40$, $p = .05$, $n = 37$). These findings suggest that there may be complex interrelationships between opiate substances in CSF and different measures of acute and chronic anxiety in normal volunteers and depressed patients.

CSF beta-endorphin measured by radioimmunoassay was also not significantly different in unipolar and bipolar depressed patients compared to manic patients or normal volunteers. Preliminary evidence suggested that CSF immunoreactive beta-endorphin was differentially related to personality characteristics in female compared to male volunteers.

c. Somatostatin in CSF: CSF somatostatin has been measured in the CSF of affectively ill patients and normal volunteers by sensitive radioimmunoassay in collaboration with Drs. D.R. Rubinow, S. Reichlin, and P.W. Gold. Dr. Rubinow found that CSF somatostatin was significantly decreased in depressed patients compared to those re-studied in the euthymic state or compared to normal volunteer controls. These findings replicate those of Gerner et al. of state-related decreases in CSF somatostatin in depression. There have now also been three additional replications. CSF somatostatin in affectively ill patients was significantly and inversely correlated with the number of hours of sleep in the night prior to the LP. These data are consistent with those in the animal literature that somatostatin decreases a variety of sleep parameters including total sleep. As noted above, carbamazepine significantly decreased CSF somatostatin, while other psychotropic drugs produced no significant alterations and the relatively specific blocker of serotonin reuptake, zimelidine, significantly increased CSF somatostatin. These findings thus open new areas for exploration of the possible role of somatostatin decreases in depression, relative increases in relationship to degree of sleep disturbance, and in the possible mechanism of action of carbamazepine, which has an interesting spectrum of clinical efficacy in affective illness, seizure disorders, and paroxysmal pain syndromes.

Dr. Rubinow, in collaboration with Drs. Doran, Pickar, Roy and Paul has also found that depressed and schizophrenic patients who were cortisol hypersecretors, as indicated by escape from dexamethasone suppression, had significantly lower CSF somatostatin. Confirming evidence in vitro that somatostatin inhibits ACTH secretion (Reisin and Axelrod), this study provides one of the first biochemical markers for cortisol hypersecretion in depression.

d. CRH, ACTH, and Cortisol: Drs. P.W. Gold and D.R. Rubinow have extensively studied pituitary-adrenal dysregulation in affective illness. They have observed significantly higher excretion of urinary free cortisol in unipolar and bipolar depressives compared to normal volunteers, with significantly lower levels in manic patients. These findings are paralleled by a large literature of well-documented and replicated studies indicating that approximately 50% of depressed patients show evidence of cortisol hypersecretion measured either by escape from dexamethasone suppression, increased urinary free cortisol, or altered diurnal variation of cortisol secretion. In addition, Dr. Rubinow has documented marked state-related alterations in urinary free cortisol secretion

and highly significant correlations in 8 AM plasma cortisols with severity of depression in cycling manic-depressive patients studied longitudinally. In our studies of urinary free cortisol and in the literature on dexamethasone suppression, severity of depression has not been well correlated with evidence of pituitary-adrenal axis disinhibition. It was particularly noteworthy to find that patients with higher levels of urinary free cortisol showed greater cognitive impairment on the Halstead Categories test of abstracting ability ($r = .48$, $p = .01$). These findings suggest that patients with higher levels of urinary free cortisol are more cognitively impaired, which is of interest in relationship to the high density of glucocorticoid binding sites measured in limbic structures, such as the hippocampus, which are thought to be critically involved in some aspects of learning and memory function. It is possible that either the high levels of cortisol or the neurochemical alterations underlying this abnormality are associated with this objective measure of cognitive impairment. These data are of some theoretical relevance, as well as of possible clinical significance, since depressed patients often have marked complaints of subjective decreases in cognitive and memory capacity.

Dr. Gold has completed a series of studies of CRH infusions in affectively ill patients and controls (as described in Project # Z01 MH 00452-09 BP) and found evidence for blunted ACTH response in depression but not manic or improved states. In contrast to depressed patients, hypercortisolemic patients with Cushing's disease show ACTH hypersecretion to CRF, providing a possible differential diagnostic test. Dr. Gold, in collaboration with Dr. Chrousos, has been recognized for this pioneering work with the receipt of the C. Richter Prize in Psychoneuroendocrinology.

e. Vasopressin and Oxytocin: Vasopressin and oxytocin are of considerable interest since a large body of experimental data in animals and preliminary studies in man suggest that they may have effects on learning and memory. Vasopressin has been measured in plasma and CSF in collaboration with Drs. P.W. Gold, D.R. Rubinow, and G. Robertson. Dr. Gold has observed that CSF values in non-psychotic bipolar depressed patients were significantly lower than those in the manic phase of the illness. In contrast, CSF oxytocin, measured in collaboration with Dr. D. Fisher, was found by Dr. Gold to be significantly decreased in manic patients compared to normal volunteers. Preliminary evidence suggests that vasopressin may be secreted directly into CSF independently of alterations in its peripheral levels. These and related data suggest that study of peptides in CSF may provide useful indirect markers of CNS peptide function and provide a basis for studying alterations in relationship to a variety of neuropsychiatric symptoms and syndromes. Drs. Rubinow, Gold, and Weingartner are studying the effects of infused oxytocin and vasopressin on mood and cognitive capacities of affectively ill patients and normal volunteers. Initial data suggest that vasopressin enhances, while oxytocin impairs, certain aspects of cognition and that vasopressin increases cortisol secretion. A vasopressin binding site on human platelets has been tentatively identified in studies in collaboration with Dr. Berrettini. These studies raise the possibility that one may be able to indirectly measure vasopressin receptor function in man in addition to other measures, such as that of vasopressin itself in CSF.

5. Life Charting the Course of Affective Illness

In collaboration with Dr. P. Roy-Byrne, Dr. T.W. Uhde, T. Porcu and D. Davis, we have recently completed the first phase of analysis of the life course of illness in 95 unipolar and bipolar patients. In addition to this detailed retrospective life chart evaluation, cyclicity within NIMH has been precisely characterized and provides a partial validation of the retrospective data. Specifically, many of the characteristics observed prior to NIMH admission, such as rapidity of cycling, are highly correlated with those observed prospectively on our clinical research unit. The recurrent nature of affective illness in our patient population is again emphasized by the finding that 80% of the patients relapsed within five years of their first episode. Fifty-five percent relapsed within the first year of that episode. The hazards of making a diagnosis of unipolar depression are also reemphasized in our sample, as we have found that eight of 23 bipolars (34%) had not yet had their first manic episode after three or more depressive occurrences. However, 21 of 23 depressed patients (92%) had converted to bipolarity after six depressive episodes. We also found that a shorter well interval between the first and second episode predicted an increased number of total weeks ill subsequently. Several mood phases or switches within the first episode, as opposed to an isolated mania or depression, also predicted increased total number of weeks ill. Bipolar patients who presented with a first depression showed a relatively greater proportion of depressions to mania subsequently, tended to have a greater proportion of depressions preceding mania, and were more likely to become rapid cyclers. Twenty-four of 46 (56%) bipolar patients showed a pattern of sensitization characterized by an increasing rapidity of cycling and decreased well interval between successive episodes of affective illness. Those who showed this sensitization pattern compared to those who showed relatively short well intervals from the onset of their illness had a significantly higher percentage of depressive compared to manic episodes and showed an increased frequency of hospitalizations for depressions. They also had significantly more episodes in the year prior to NIMH admission.

It was particularly noteworthy that while bipolar patients had increased numbers of total and depressive episodes, increased episodes per years ill, and increased episodes in the year prior to NIMH admission, they showed an equal amount of total time ill compared to unipolar patients. This emphasizes the finding that bipolar patients tend to have more episodic and cyclic courses to their illness yet may show similar durations of impairment compared to the unipolars. Few characteristics of bipolar illness differentiated those with early compared to late onset. Those with late onset had a longer first episode of illness.

Interesting findings did emerge comparing bipolar female and male patients. Females showed an increased number of hospitalizations for depression. Moreover, the 34 bipolar females showed a pattern of more rapid cycling (5.8 ± 1.1 episodes in the year prior to NIMH admission) compared to the 23 male bipolars who only showed 2.7 ± 0.5 episodes prior to NIMH admission ($p = .04$). These data are consistent with other studies in the literature suggesting that female patients are more prone to rapid cycling illness and are of considerable interest in relation to theories of underlying neurotransmitter and hormonal alterations in females compared to males.

Rapid compared to less rapid cycling bipolar patients showed an increased number of weeks ill in the year prior to NIMH hospitalization as well as increased number of hospitalizations for depression. The occurrence of rapid cycling also predicted an increased number of weeks hospitalized at NIMH (43 wks compared to 27 wks for non-rapid cyclers) as well as more manias and total episodes of affective illness observed at NIMH. Thus, rapid cycling prior to NIMH admission correlated with a pattern of continued rapid cycling during NIMH admission.

Thus, the study of the longitudinal course of affective illness provides a template not only for assessing the phenomenology of the illness and its response to treatment interventions with agents such as lithium and carbamazepine, but also refocuses on possible biological mechanisms underlying the recurrent and, at times, progressive aspects of affective illness. For example, we have noted above findings of increased noradrenergic function in depression associated with rapidity of cycling. Studies in laboratory animals of behavioral sensitization to stimulants and stressors and of electrophysiological kindling may provide insights into different types of mechanisms underlying the progressive evolution of behavioral disturbances in response to repetition of the same stimulation over time.

We suggest that the life charting process is a useful clinical as well as research tool and may help focus on possible environmental precipitants and dynamically significant events and stresses that may be temporally related to affective episodes. It also allows precise characterization of the degree of longitudinal response to newly available pharmacological agents. Recent data of Wehr and Goodwin have emphasized that some pharmacological interventions such as the tricyclic antidepressants may actually result in increased rapidity of cycling. The life chart methodology provides a useful instrument for following this problematic side effect.

6. Menstrually-Related Mood Dysfunction (Dr. D.R. Rubinow)

A relationship between mood and behavior and menstrual function has been described with respect to a number of disorders including premenstrual tension, post-partum depression, epilepsy (so-called catamenial epilepsy), and menopausal dysphoria. Dr. D.R. Rubinow has initiated a series of studies to investigate the relationship between mood disorders and the menstrual cycle (see project #Z01 MH 00180-02 BP). These studies include: development of a questionnaire which is being employed to help determine the incidence and nature of affective symptoms in relation to the menstrual cycle; assessment of the precision of the relationship between mood changes and the menstrual cycle utilizing daily self-ratings and daily temperature recordings; investigation of hormonal activity employing periodic blood samples and neuroendocrine tests; and assessment of the efficacy of progesterone, a synthetic progestin, and carbamazepine in the treatment of established menstrually-related mood syndromes. The results of such a study may: 1) determine whether a specific association between depressive symptoms and menstrually-related phenomena (menstruation, post-partum depression, menopause, hormone-induced behavioral change) can be established; 2) reveal the incidence of the entrainment of depressive symptoms to the menstrual cycle; 3) help elucidate the nature of the "switch" mechanism in affective disorders and

periodic psychosis; and 4) determine the efficacy of pharmacologic agents believed useful in the treatment of menstrually-related mood disorders.

7. Depressive Subtypes and Symptoms in Relation to Regional Localization of Function

a. Atypicality of Depression: In collaboration with Dr. E.K. Silberman, we have devised an atypicality of depression rating scale in order to more precisely characterize the range of atypical depressive presentations in patients who otherwise meet formal Research Diagnostic Criteria for primary affective illness. Older patients and those with bipolar I affective illness had more typical presentations than those of unipolar or bipolar II patients. The more typical patients showed increased rapid cycling in the year prior to NIMH admission although, interestingly, decreased numbers of total hospitalizations compared to the atypical patients. Atypical depressed patients also showed more variance in biological measures such as those of the noradrenergic system, further suggesting that the range of clinical presentations may be related to the range of biological variables that have been hypothetically linked to depressive illness. It was of interest that both typical and atypical depressed patients showed similar degrees of cortisol hypersecretion.

b. Psychosensory Phenomena: In collaboration with Drs. E.K. Silberman and J-P. Boulenger we have developed an interview rating scale designed to measure signs and symptoms that are usually associated with psychomotor epilepsy (complex partial seizures). We have studied these phenomena in patients with primary affective illness without evidence of seizure disorders, in patients with documented evidence of temporal lobe epilepsy, and in a medical control group of hypertensive patients. Compared to the control group, patients with affective illness and with epilepsy showed a highly significant increased incidence in the number of these signs and symptoms. To the extent that psychosensory distortions and related symptoms usually associated with temporal lobe epilepsy are occurring with a high incidence in patients with primary affective illness, it might suggest that some of the neural substrates involved in complex partial seizures overlap with affective illness. Patients with greater numbers of psychosensory symptoms responded better to lithium carbonate, and preliminary data suggest that this is not the case for carbamazepine, as we would have predicted.

c. Psychological, Structural, Metabolic, and Electrophysiological Approaches to Regional Brain Function in Affective Illness: A variety of psychological test batteries are employed to assess possible alterations in regional brain function in patients with affective illness, including the Luria Battery, the Halstead Categories Test, tachistoscopic presentation to assess hemispherical laterality, and other cognitive tests studied in collaboration with Dr. E.K. Silberman and K. Squillace of the Laboratory of Psychology and Psychopathology. Consistent with patients' subjective sense of cognitive impairment during depression, marked impairment in cognitive function has been documented on the Halstead Categories Test.

Degree of cognitive dysfunction correlated with increases in urinary free cortisol secretion, suggesting that cortisol hypersecretion may be primarily or

secondarily related to this important subjective and objective deficit in depressed patients. The Luria Battery provides another approach to assessment of possible regional areas of dysfunction and has been completed in more than 45 patients.

Computerized axial tomography (CAT) scans have been performed on our patients with affective illness and reveal a similar range of increased ventricular brain ratios (VBRs) comparable to those observed in schizophrenic patients. We are currently assessing the clinical and biological concomitants of this evidence of altered brain structure in a subgroup of affectively ill patients with Dr. C.H. Kellner. Dr. Kellner observed that patients with the greatest urinary free cortisol excretion had the largest VBRs ($r = .81$, $p = .02$). These data are consistent with those in the literature indicating that treatment with ACTH or exogenous glucocorticoids such as dexamethasone is associated with reversible atrophy and enlarged ventricles on CAT scan. This literature and our findings suggest that "structural" alterations in brain on the CAT scan may not be as irreversible as previously thought and that exogenous and perhaps endogenous biochemical changes may be important mediators of this brain measure which is receiving increasing attention as a possible concomitant of some patients with schizophrenic illness.

Twenty-one medication-free affectively ill patients also showed a significant relationship between VBR and degree of cognitive impairment measured on the Halstead Categories Test (with Drs. Kellner and Rubinow). As described in detail elsewhere, topographic mapping of EEG frequencies and averaged evoked response is being conducted in collaboration with Drs. R. Cohen, L. DeLisi, H. Holcomb and M.S. Buchsbaum. These studies, in conjunction with positron emission tomography (PET) scan studies, may provide important evidence of electrophysiological and/or metabolic regional dysfunction in affective illness. These findings can then be compared with ongoing psychological, longitudinal, physiological, and biochemical assessment of affectively ill patients in order to complete a coherent and comprehensive assessment of possible interrelationships of these measures in affective illness. Initial studies indicate that acutely ill and improved affective disorder patients show a nonspecific pattern of hypofrontality similar to that observed in schizophrenia and other patient populations. These and other data indicate that relative hypofrontality in glucose utilization is not specific to the psychopathology of schizophrenia and other alterations more intimately related to affective symptomatology remain to be elucidated.

Dr. DeLisi has also measured glucose utilization in temporal cortex relative to other areas in the same brain slide and found it to be lower in depressed patients compared to controls. These data are of particular interest in relationship to our finding of the efficacy of the anticonvulsant carbamazepine in affective illness. They provide evidence that depressed patients are not showing areas of increased glucose utilization in the temporal lobe similar to that observed in epileptic patients with complex partial seizures (Engel et al., 1982), suggesting that carbamazepine is not acting by dampening covert seizure activity in affective illness.

d. Procaine Infusions as a Probe of Limbic System Responsivity:
Graded doses of the local anaesthetic procaine were administered to affectively

ill patients (in collaboration with Drs. C. Kellner, F. Putnam and B. Vittone), borderline personality disorders (in collaboration with R. Cowdry and D. Gardner) and normal volunteers in an attempt to probe limbic system responsivity. Analysis of the first 21 subjects by Dr. R. Coppola reveals selective increases in fast activity, especially 45 Hz, in temporal cortex, confirming in man suggestions from animal studies that local anaesthetics activate temporal lobe and limbic structures based on depth electrode and deoxyglucose studies. These studies are paralleled by analyses by Dr. R. Adamec also indicating omega band activity in anterior temporal leads following procaine.

Dose-related alterations in subjective sensory and cognitive functions were reported as well as a variety of affective responses ranging from mood elevation to dysphoria. Vivid recall of experientially immediate memories, as well as hallucinatory-like phenomena, occurred less often. Preliminary analysis suggests that patients with borderline personality disorder may be more responsive to the sensory and affective changes induced by procaine compared to affectively ill patients or normal controls.

In collaboration with Drs. P. Gold and C. Kellner, procaine-induced release of ACTH, cortisol, and prolactin, but not growth hormone, has also been documented. Interrelationship between sensory, affective, electrophysiological and endocrine changes are being examined.

8. Laboratory Studies of Behavioral Sensitization and Electrophysiological Kindling

a. Stimulant-induced Behavioral Sensitization: A series of studies have investigated the mechanisms underlying increased behavioral responsivity to the same dose of a psychomotor stimulant such as cocaine. Animals administered cocaine (10 mg/kg i.p.) once-daily showed increasing amounts of locomotor hyperactivity and stereotypy to the same dose over time. An environmental context and conditioning component has been demonstrated. Animals repeatedly treated with cocaine in the context of the test cage showed greater degrees of hyperactivity and stereotypy than animals receiving identical doses in a different environment. Brattleboro homozygote rats lacking vasopressin showed deficient onset, maintenance, and persistence of cocaine-induced behavioral sensitization compared to their heterozygote litter-mate controls. We have replicated the original findings and further show that vasopressin replacement will reverse the deficit in cocaine-induced behavioral sensitization. Female, compared to male, rats are more responsive to the same dose of cocaine. They demonstrate similar behavioral sensitization to repeated injections of cocaine at approximately half the dose (5 mg/kg) used in males (10 mg/kg, i.p.).

Studies in collaboration with Dr. K. Zander have assessed cross-sensitization between cocaine-induced hyperactivity and several types of stresses such as those induced by tail pinch. It appears that type of stress, its intensity, and longitudinal time course are important determinants of whether animals will show increased or decreased responsivity to a cocaine challenge. Some aspects of the response to repeated stress showed clear-cut sensitization effects, while others appeared to show adaptation or tolerance. A 40 kHz vocalization showed increasing amplitude of response to repetition of the same level of tail pinch stress over time.

b. Electrophysiological and Chemical Kindling: Repeated, intermittent electrical stimulation of the brain results in increasing duration, spread, and complexity of electrical after-discharges culminating in the appearance of major motor seizures to a previously subthreshold stimulation. We have employed this procedure, as described by Goddard et al., in order to study long-lasting changes in neural and behavioral excitability that accompany this process. Following electrical kindling of the amygdala, rats showed decreased spontaneous and cocaine-induced exploratory activity, while they showed increased convulsive susceptibility to a related local anaesthetic, lidocaine. Repeated injections of the same dose of lidocaine (65 mg/kg, i.p.) lead to an increasing incidence, severity, and duration of seizures to the same dose over time. This effect does not appear to be a pharmacokinetic one, as blood levels of lidocaine and its metabolite are not increased with chronic administration. Moreover, if lidocaine-induced excitability and seizures are blocked by the co-administration of diazepam, no seizure sensitization occurs. Finally, repeated lidocaine-induced seizures sensitize to electrophysiological kindling of the amygdala such that amygdala-kindling proceeds three times faster following lidocaine pretreatment compared to saline controls. These data suggest some degree of cross-sensitization between electrical and chemical modes of kindling.

Behavioral alterations persist in the interictal period following lidocaine-induced seizures. In collaboration with Drs. L. Sokoloff, C. Kennedy and their associates in the Laboratory of Neurochemistry, it has been demonstrated that lidocaine-induced seizures relatively selectively increase metabolic activity in limbic system structures, particularly amygdala, hippocampus, perirhinal, and cingulate cortical areas. It is, thus, of interest that increases in irritability and resistance to capture are prominent following lidocaine seizures but not following seizures induced by electroconvulsive shock or pentylenetetrazol (Metrazol). The changes in irritable and aggressive behavior following lidocaine seizures persist for some days into the interictal period. This paradigm would therefore appear to be a useful one in exploring the relationship of seizures with some specificity for limbic structures to alterations in aggressive behavior.

Studies with Dr. S.R.B. Weiss have shown that carbamazepine (15 mg/kg, i.p.) is a potent inhibitor of completed amygdala-kindled seizures, but at this dose is not effective in suppressing the development of kindling in the rat. As noted above, we have recently observed that carbamazepine-10,11-epoxide is more highly correlated with the degree of psychotropic response in our patients than is the parent compound. We have demonstrated that the metabolite carbamazepine-10,11-epoxide is also effective in inhibiting amygdala-kindled seizures, although it is slightly less potent than carbamazepine itself. Possible mechanisms underlying the kindling process itself are being studied in collaboration with Drs. J. Patel and P. Marangos. Preliminary evidence has been obtained indicating that 24 hours following kindling there is selective phosphorylation of a 45K protein in the amygdala bilaterally; it is not observed following repeated ECT seizures. Dr. Patel has extended these findings with the observation of regional changes in phosphorylation. He found changes in a calcium-calmodulin sensitive 35K protein bilaterally in the amygdala of kindled rats. Additional proteins, including an 87K protein (SMP) and an 80K (synapsin) also appeared to be increased by kindling. These changes were not observed with lidocaine-induced seizures, suggesting

some specificity to the process and raising the possibility of dissection of more molecular mechanisms involved in kindling.

c. Electroconvulsive Shock Inhibits Amygdala Kindling: The clinical utility of an anticonvulsant such as carbamazepine appears paradoxical in relation to the use of electroconvulsive therapy (i.e., major motor seizures) in the treatment of both manic and depressed phases of affective illness. One possible explanation of this paradox emerges from two separate studies, in collaboration with Dr. F.W. Putnam and N. Contel, demonstrating that the major motor seizures of electroconvulsive shock (ECS) are themselves anticonvulsant to amygdala-kindled seizures. Pretreatment with ECS six hours prior to amygdala kindling markedly inhibits development of kindled seizures compared to sham ECS or compared to ECS administered immediately after kindling. In a second study, we used a more clinically relevant paradigm. Animals were kindled to their first stage for major motor seizure and then were treated with single or seven daily ECS or sham ECS. Following this seven-day interval, amygdala kindling was resumed. Chronic ECS, but not one ECS followed by a seven-day delay, markedly inhibited amygdala-kindled seizures for up to five days compared to sham ECS controls. Taken together, these two studies indicate that the major motor seizures of ECS can, in two different time frames, exert marked anticonvulsant effects on amygdala-kindled seizures. These data raise the possibility that the efficacy of electroconvulsive therapy in patients with affective illness could be related to effects mediating its anticonvulsant actions.

d. CRF Seizures and Behavior: Interaction with Amygdala Kindling: Dr. S.R.B. Weiss, in collaboration with Drs. A. Pert and P. Gold, has conducted a series of studies on the behavioral and convulsive effects of corticotropin releasing hormone (CRF) administered intracerebroventricularly. CRF induces the late onset (i.e., following a lag 4-6 hours post injection) of seizures that resemble behaviorally and electrophysiologically those produced from electrical stimulation of the amygdala.

Following repeated once-daily administration, tolerance develops to the effects of CRF on seizures. Despite this, CRF seizures produce cross-sensitization to amygdala kindling. That is, animals pretreated with CRF develop seizures electrically kindled from the amygdala twice as fast as vehicle-injected controls. CRF-treated animals also show increases in aggressive behavior toward other rats (conspecifics).

The convulsive and aggressive behaviors were not reliably reproduced by local intracerebral injection of CRF into amygdala, hippocampus, septum, hypothalamus, or PAG. Moreover, lesions of the amygdala, hippocampus, or olfactory tubercle did not block the development of seizures produced by i.c.v. CRF. These data suggest that CRF is inducing seizures highly similar to those produced by electrical stimulation of the amygdala, but that they are not dependent on an amygdala substrate for their occurrence. Further, these data suggest the possibility that an endogenously produced, stress-related peptide such as CRF may, under pathological conditions, be associated with alterations in convulsive and aggressive responsivity. The relationship of neuropeptides to convulsive phenomena deserves further experimental exploration, particularly in light of the production of limbic seizures with some opiate peptides and the recent observations linking somatostatin decreases to anticonvulsant activity.

D. Proposed Course of Project

As carbamazepine is emerging as an effective treatment modality in some patients with manic-depressive and schizoaffective illness, we will attempt to further delineate clinical and biological markers of carbamazepine response. Preliminary evidence suggests that many patients who clearly do not respond to lithium carbonate will respond to carbamazepine. It will be increasingly important to establish whether response to carbamazepine compared to lithium carbonate delineates separate subgroups of patients with affective illness. It is also possible that carbamazepine may be more effective in later stages of the illness, particularly when the patients are in a treatment-resistant, rapid-cycling phase of illness. The degree of generalization of carbamazepine response to other anticonvulsant agents such as phenytoin or valproic acid will be another area of both clinical and theoretical import. This is also particularly the case in light of our recent finding that electroconvulsive therapy may be exerting potent anticonvulsant effects on limbic system seizures. Are anticonvulsant effects of a variety of treatment modalities linked to therapeutic response in affective illness? Carbamazepine is clearly useful in pain syndromes that do not involve a convulsive process, and effectiveness of anticonvulsant agents in a subgroup of patients with affective illness does not imply an underlying ictal process. The possible mechanisms of action of carbamazepine studied in our clinical population, as well as in behavioral pharmacological models and at more basic molecular levels, will also be pursued.

Topographic mapping of electroencephalographic activity and PET scan techniques will be explored in collaboration with Drs. R. Cohen, H. Holcomb, L. DeLisi and M.S. Buchsbaum, not only in affectively ill patients compared to controls, but also as they might predict or correlate with treatment response. Further clinical and laboratory work will be pursued to investigate whether carbamazepine's anticonvulsant metabolite carbamazepine-10,11-epoxide has active psychotropic properties.

The interrelationship of classical neurotransmitter substances with the putative CNS neurotransmitter peptides will be explored in both patients with affective illness and anxiety disorders, in collaboration with Drs. D.C. Jimerson and T.W. Uhde. A variety of techniques are in place for measurement of neurotransmitter and receptor function in both classical neurotransmitter systems and in the peptide systems in man. These will be correlated with behavioral alterations and changes in mood and cognitive functioning in patients with mood and anxiety disorders.

Particular focus will be given to studies of CRF to elucidate its utility in the differential diagnosis of cortisol hypersecretion of Cushing's disease and depression. Alterations in somatostatin as they relate to affect and seizure mechanisms will also be systematically explored.

As described in detail in Project # Z01 MH 00071-04 BP, Dr. T.W. Uhde will continue to explore the similarities and differences in patients with panic anxiety syndromes and those with affective illness in terms of acute symptomatology, longitudinal course of illness, and response to pharmacological agents. Catecholamines appear to be altered in both the mood disorders and in panic

anxiety disorders. Response to treatments which act on catecholamine systems such as clonidine will be compared and contrasted in both patient populations. The clinical utility of carbamazepine will also be explored in this syndrome. Dr. F.W. Putnam will complete some phases of his studies of psychological, psychophysiological, and neural mechanisms underlying patients with multiple personality syndrome and initiate others. An extensive questionnaire for 100 patients has been completed which better delineates symptoms and course of illness of multiple personality syndrome, and further supports the important etiological role of childhood physical and/or sexual trauma (see Project # Z01 MH 00072-04 BP).

Dr. D.R. Rubinow is also continuing to develop a new combined inpatient and outpatient focus on patients with menstrually-related exacerbation of mood and behavior disorders. He will be examining this problem from a clinical and endocrinological point of view, and as a model for studying the acute onset and offset of affective dysfunction. Similar studies will be pursued utilizing sleep deprivation, which represents another non-pharmacological means of inducing rapid and non-pharmacologically related improvement in mood, as well as examining mechanisms that may underlie exacerbation of depression that occurs regularly when patients return to sleep.

Work in animal models will continue to focus on possible mechanisms underlying behavioral sensitization and electrophysiological kindling. In collaboration with Drs. P. Marangos and J. Patel, neurotransmitter receptors, protein phosphorylation, and ion channels will be examined as possible mediators or modulators of the electrophysiological kindling paradigm. Studies of behavioral and biochemical response to repeated stress (avoidable and unavoidable) will be performed in collaboration with Drs. S.R.B. Weiss and A. Pert. The role of environmental context and conditioning will also be examined in these paradigms.

E. Significance to Biomedical Research and the Program of the Institute

Findings in several research areas are of considerable clinical and theoretical significance. Carbamazepine has emerged as a new treatment for manic-depressive illness; it is effective in some patients who do not respond to lithium carbonate. In addition, clinical and basic work exploring the mechanism of action of this compound alone or in comparison to lithium carbonate and other clinically effective psychotropic agents may provide new leads to the understanding of mechanisms of action of effective antimanic and antidepressant drugs and mechanisms underlying affective dysregulation. Study of endocrine and peptide substances in man and animals may also provide new conceptual and practical approaches to the relationship between manic and depressive symptoms and biochemistry. Examination of the interaction between classical neurotransmitters and the peptides should prove fruitful in understanding normal and pathological functioning. The multi-disciplinary assessment of our patients' mood, behavior, cognition, physiology, and biochemistry will allow more precise characterization of important biobehavioral relationships and their underlying neural substrates. Study of the mechanisms underlying behavioral sensitization and kindling should yield important information regarding the coding of long-term changes in the CNS. Thus, basic and clinical research have led to important findings in neurobiology and the development of a new treatment for affective illness with carbamazepine. Last year, Drs. Uhde and Post received the Biological Psychiatry Society and

American Psychiatric Association's research prizes, respectively, for their clinical research studies, and this year Dr. Gold was awarded the C. Richter prize for Psychoneuroendocrinology, emphasizing the wide import and recognition of the work in this clinical group.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

201 MH 00072-04 BP

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Psychophysiological Investigation of Multiple Personality Disorder

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Frank W. Putnam, M.D., Staff Psychiatrist, Adult Psychiatry Branch, NIMH
 Dr. Richard Coppola, Laboratory of Psychology & Psychopathology, NIMH
 Dr. John Bartko, Div. of Biometry & Epidemiology, NIMH
 Dr. Theorode Zahn, Laboratory of Psychology & Psychopathology, NIMH
 Dr. John Morihisa, Adult Psychiatry Branch, NIMH

COOPERATING UNITS (if any)

Adult Psychiatry Branch, Laboratory of Psychology & Psychopathology, Division of
 Biometry & Epidemiology, National Institute of Mental Health

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Section on Psychobiology

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda M.D., 20205

TOTAL MAN-YEARS:

2.0

PROFESSIONAL:

2.0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project focuses on the psychophysiological differences manifest among the alternate personalities of patients with multiple personality disorder (MPD). The occurrence of psychophysiological differences among alternate personalities in excess of chance levels has been documented for EEG, visual evoked potentials, and measures of autonomic activity including heart rate, respiration, skin temperature, and bilateral galvanic skin response. In most cases these differences significantly exceed those manifest by control subjects who were simulating alternate personalities. In some instances, however, control subjects demonstrate as much variability as MPD patients. Further studies are underway to elucidate the mechanisms of these psychophysiological changes.

Psychophysiological Investigation of Multiple Personality DisorderI. Project DescriptionA. Objectives

This study is part of a multi-disciplinary effort to investigate and rigorously document clinical and psychophysiological phenomena associated with multiple personality disorder and related dissociative states.

B. Methods1. Subjects

a. Patients who meet DSM-III criteria for multiple personality disorder are admitted as outpatients to the NIH Clinical Center under protocol #80-M-142. These patients serve as subjects in the physiological investigations.

b. Clinical Center normal volunteers and professional actors affiliated with the Psychodrama Institute located at St. Elizabeth's Hospital in Washington, D.C. serve as control subjects for the physiological studies.

2. Investigations

a. Drug-free subjects receive a repeated series of EEG trials in collaboration with Dr. John Morihisa of the Adult Psychiatry Branch. These trials are analyzed with the topographical spectral analysis software developed by Dr. Richard Coppola of the Laboratory of Psychology, and statistical differences between the groups are determined using the SAS and BMDP software available through the Division of Computer Resources and Technology in consultation with Dr. John Bartko of the Division of Biometry and Epidemiology.

b. Autonomic measures including bilateral galvanic skin response (GSR), heart rate, respiration and temperature are studied across a number of resting and stimulus paradigms in collaboration with Dr. Theodore Zahn of the Laboratory of Psychology.

II. Major Findings

A. EEG and Evoked Potential Studies. Results of the EEG investigations are included in a project report from the Adult Psychiatry Branch.

B. Autonomic Studies. Results of the autonomic measures indicate that 8 of the 9 multiple personality subjects measured exceeded the "chance" number of significant effects and showed clearly differentiated physiological patterns.

In the control group, 3 out of the 5 subjects analyzed to date exceeded the "chance" levels. Two of these three subjects manifested these significant differences between a hypnotized and non-hypnotized state. Two of the three non-hypnotized controls were not able to produce consistent interpersonality (or

interstate) physiological differences at or above chance levels and had fewer significant differences than any multiple personality subject.

III. Summary

The multiple personality patients as a group showed clearly differentiated physiological patterns. Some control subjects, with the aid of hypnosis, were able to produce differentiation as great as the "better" patients. However, analysis of the data suggests that the hypnotized controls had very low arousal compared to the "main" personality, while the psychophysiologically distinct alternate personalities of the multiple personality patient group had increased arousal compared to the "main" personality. This suggests that the two groups were producing their physiological changes through different mechanisms. Further investigations to elucidate the nature of these differences are underway.

IV. Significance for Biomedical Research

Multiple personality disorder is an unusual psychopathological disorder. There is very good evidence to link the development of this disorder to a childhood history of severe sexual and/or physical abuse, usually occurring between ages 2 and 12. The dissociated states, which seem to initially serve as buffers to contain the emotional experience of abuse for the child, develop over time into a series of separate and distinct personalities which exercise semi-autonomous control over the individual's behavior. The focus of this project is on the delineation and documentation of psychophysiological differences among the alternate personalities of individuals with multiple personality disorder. Elucidation of the mechanisms involved in this disorder may shed light on the interactions of personality factors and psychosomatic phenomena in general, and on the processes by which extreme trauma produces somatic disturbances such as those frequently found in post-traumatic stress disorders.

Publications:

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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00071-04 BP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Psychobiological Correlates and Treatment of Panic and Related Mood Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

T.W. Uhde, M.D., Chief, Unit on Anxiety and Affective Disorders, BPB, NIMH

COOPERATING UNITS (if any)

Outpatient Department, NIMH; CPB, LPP, NS, LCS, NIMH; NIAAA; VA Medical Center, Bronx, N.Y.; University of California at Irvine; University of Oregon; San Diego Veteran's Medical Center

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Section on Psychobiology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

5

PROFESSIONAL:

4

OTHER:

1

CHECK APPROPRIATE BOX(ES)

 (a) Human subjects (b) Human tissues (c) Neither (a1) Minors (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Patients with pathological degrees of anxiety who meet DSM III criteria for generalized anxiety, panic or phobic disorders are evaluated using psychological, physiological, and biochemical methodologies. Patients with major affective illness, particularly those with a significant anxiety component, are also eligible for participation in the program. Particular attention is given to the role of the noradrenergic neurotransmitter system as assessed by: 1) measurement of the metabolite MHPG in urine, plasma, and CSF; 2) adrenergic receptor number and function in platelets; and 3) neuroendocrine and behavioral response to the alpha-2 adrenergic agonist clonidine, and antagonist yohimbine. Research investigating the relationship of noradrenergic function to other neurotransmitter systems such as those which influence opiate, adenosine, and GABA-benzodiazepine function also have been initiated. Caffeine challenges are administered to assess its behavioral and biochemical effects. Other approaches to understanding the pathophysiology of anxiety and its potential treatment with alprazolam, carbamazepine, clonidine, imipramine and propranolol will be explored.

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I. Project Description

A. Objectives

This project employs a multidisciplinary team in the study and treatment of pathological anxiety, major affective and related mood disorders.

B. Methods Employed

1. Subjects

a. Patients who meet Research Diagnostic Criteria for panic, phobic, and generalized anxiety disorders as well as patients who meet DSM III criteria for major affective illness are candidates for participation in the project. Inpatients are studied and treated on the 3-West Clinical Research Unit and outpatients are followed through the Ambulatory Care Research Facility. A number of previously validated scales to measure state and trait anxiety are utilized and an analogue anxiety scale and panic anxiety scale have been developed to more adequately assess the relationship among state anxiety, phobic anxiety, avoidance behavior, and depressive symptomatology.

b. Normal volunteers are also accepted into the project to provide control data as well as to assess the relationship between normal state anxiety and selected psychological and biological variables.

2. Psychological and Biological Evaluation

a. Baseline Evaluation. During an initial evaluative period patients undergo extensive neurological, psychological, biochemical, and neurophysiological evaluation. This initial evaluation is indicated due to the heterogeneous nature of the panic and phobic disorders. Anecdotal reports suggest that many medical illnesses may present as or exacerbate pre-existing conditions of pathological anxiety. However, no research has systematically studied a large number of panic and phobic patients to determine the incidence and prevalence of these associated disorders.

b. Life Chart Methodology. A life chart technique has been developed in collaboration with Dr. P. Roy-Byrne, M. Geraci, and Dr. J.-P. Boulenger, to plot the frequency, intensity, and interval between panic attacks. This approach will allow us to document the development, recurrence, and progression of the panic and phobic disorders. Life charting is an important aspect of the overall project because few systematic studies have been conducted on the natural progression of these disorders.

c. Sleep Research. Electroencephalographic sleep recordings are obtained for three consecutive nights. Although many panic anxious patients, like endogenously depressed individuals, have improved sleep following treatment with tricyclic and monoamine oxidase inhibitors, little is known about the sleep architecture of panic and phobic anxious patients. In collaboration with Drs. J.C. Gillin and W. Mendelson, this ongoing research represents the first attempt to our knowledge to evaluate the sleep profile of this patient population.

- d. Insensitivity Index. Using threshold and signal detection methodology, an index of pain insensitivity is obtained in patients and normal volunteers following the intravenous administration of clonidine 2 µg/kg and placebo.
- e. Galvanic Skin Response. The effects of alprazolam and imipramine and selected standard anxiolytics on physiological measures of galvanic skin response, reaction time to auditory tones, pulse, and respiratory rate are studied in panic and phobic anxious patients and age-matched normal volunteers. This investigation is performed in collaboration with Dr. T. Zahn.
- f. Echocardiography. Echocardiography is obtained in patients and age-matched controls to assess the presence or absence of mitral valve prolapse. These data are obtained in collaboration with Dr. R. Watson who is blind to the diagnosis of each patient or normal volunteer when echocardiography and auscultation are performed.
- g. Computerized Axial Tomography. Cerebral CAT Scans are obtained, and, in collaboration with Dr. C. Kellner, cerebral ventricular size is determined in patients with panic disorder. Enlargement of the cerebral ventricles has been reported in schizophrenic and affectively ill patients.
- h. Caffeine. Caffeine is administered to panic patients and normal controls to assess behavioral and biochemical responses to this agent whose effects are thought to be mediated through the adenosine, GABA-benzodiazepine, and noradrenergic systems.
- i. Clonidine -- An Alpha-Adrenergic Agonist. Clonidine is administered intravenously to anxious and affectively ill patients and normal volunteers to assess clinical, physiological, and neuroendocrine responses to this noradrenergic drug.
- j. Yohimbine -- An Alpha-Adrenergic Antagonist. Yohimbine is administered in an oral challenge to panic anxious and affectively ill patients and normal controls to assess the clinical and biochemical effects of this noradrenergic antagonist which is known to potently increase noradrenergic function in the animal.
- k. Urinary MHPG and Urinary Free Cortisol. Amine metabolites and urinary free cortisol are systematically evaluated using daily 24-hour urine collections across clinical state changes on and off medication.
- l. Dexamethasone Suppression Test. Dexamethasone is administered to patients to evaluate the pituitary adrenal axis. Basal values are performed at baseline and at 8 am, 4 pm, and 11 pm following dexamethasone administration.
- m. Cerebrospinal Fluid and Plasma Studies. Amine metabolites, electrolytes, and peptides are also measured in blood and cerebrospinal fluid.
- n. Alpha-Adrenergic Receptors. In collaboration with Dr. M. Kafka, platelet alpha receptor function as well as prostaglandin-stimulated

increase in cyclic-AMP are assessed in patients and age-matched normal volunteers.

o. Melatonin. In collaboration with Dr. A. Lewy, plasma and urinary melatonin is measured during clonidine and placebo infusions. Clonidine infusions will be administered to panic anxious patients and age-matched volunteers at night during sleep.

p. Glucose and Lactate Metabolism. In collaboration with Dr. B.J. Vittone, clinical and metabolic parameters are evaluated following the oral administration of glucose.

3. Treatment

a. Psychotherapeutic. Treatment and evaluation are conducted in individual and/or group supportive sessions. In addition, ongoing clinical case conferences are utilized.

b. Routine Somatic Treatment. Both routine and experimental compounds are evaluated during double-blind clinical trials. Standard medications used for the treatment of pathological anxiety may be used and include tricyclic antidepressants, monoamine oxidase inhibitors, minor tranquilizers, and beta-blockers.

C. Major Findings

1. Medical Illnesses and Pathological Anxiety

a. Intracerebral pathology Detailed physical, neuropsychiatric, and laboratory evaluations have been performed in over 52 patients who met Research Diagnostic Criteria for panic disorder. None of these patients had known pre-existing medical illnesses that were thought to be related to their anxiety syndromes. Yet, within this group it has now been established that six patients have evidence of significant intracerebral pathology, and many more demonstrate nonspecific neurological abnormalities (e.g., cerebral atrophy on CT scan, abnormal EEG's). These preliminary findings are provocative and suggest the possibility of an increased incidence of and relationship between intracerebral pathology and some conditions of pathological anxiety.

In an attempt to further define the characteristics of brain structure in panic disorder, we have investigated, in collaboration with Dr. C. Kellner, the cerebral ventricular size (VBR) in agoraphobic patients with panic attacks. To date we have studied 35 patients. The VBR in our patients was 3.6 ± 2.5 (mean \pm S.D., range 1.0-10.8). There was significant correlation between duration of benzodiazepine use and VBR ($r = .56$, $p < .01$). Compared to published norms, these data suggest that ventricular size is normal in panic disorder patients. This negative finding in panic patients is noteworthy in relation to well-established findings in schizophrenic patients and more recent findings in affective disorder patients of increased VBR. The correlation between lifetime duration of benzodiazepine use and VBR is consistent with a recent report by M. Lader demonstrating ventricular enlargement in individuals

treated with benzodiazepines for greater than ten years. The significance of this preliminary finding is unclear, but requires further investigation.

b. Mitral valve prolapse Twenty patients have evidence of mitral valve prolapse (MVP), an abnormality previously suggested as being associated with panic attacks. The role of MVP on catecholamine function will be assessed in the near future.

c. Nonspecific illnesses Sixty-nine percent of the patients evaluated have been found to have previously undiagnosed medical illnesses apparently unrelated to the panic attacks themselves. These illnesses have ranged from mild conditions, e.g., iron deficiency anemia, to serious disorders, e.g., multiple endocrine adenomas. The reason for this high prevalence of nonspecific medical illnesses remains unclear but may be related to the nonspecific effects of stress. Several of these patients previously had received incomplete medical evaluations, perhaps in part because their physical complaints were exclusively attributed by physicians to anxiety. In addition, phobic avoidance may have contributed to a delay on the part of patients in seeking appropriate consultation and/or treatment.

Together, these preliminary data suggest that patients with severe anxiety require careful medical evaluations for underlying medical diseases, some of which may mimic or exacerbate symptoms of anxiety. Furthermore, some vulnerable patients may have panic attacks triggered by a wide range of different medical illnesses. Further research is required to determine the prevalence of endocrine, cardiovascular, and neurobiological diseases in patients with panic and other anxiety-related syndromes. When medical illnesses are present, these patients may require specialized behavioral and/or psychotherapeutic medical care. Without appropriate treatment, some patients may be overwhelmed by phobic anxiety and avoid treatment of even life-threatening illnesses.

2. Life Course of Illness

The life course of illness has been defined in 38 patients referred to NIMH for evaluation and treatment of panic attacks. Moderate to extreme agoraphobia and moderate to severe generalized anxiety were associated symptoms in 84% of patients with panic attacks. Of interest, all patients who had panic attacks without concomitant symptoms of anxiety or agoraphobia were men. Patients in this group tended to have isolated and relatively infrequent episodes of panic over a period of weeks to months followed by complete remission for months to years. We suggest, therefore, that a substantial proportion of patients with panic attacks may have time-limited periods in their lives when panic attacks may intervene. Although these individuals tended to be previously well-functioning men who developed panic attacks when exposed to major life-stress events, no clinical or biological indices are now available to distinguish between patients at low- versus high-risk for the development of pathological anxiety, agoraphobia, or depression. The identification of a biological marker for this purpose would have obvious clinical utility, including the avoidance of unnecessary treatment with medications.

Thirty of 32 patients (94%) who developed pathological degrees of generalized anxiety did so after, rather than before, the onset of their first panic

attack. Similarly, 31 of 32 patients (97%) with agoraphobia developed phobic avoidance behaviors after the onset of panic attacks. All patients with agoraphobia developed avoidance behaviors within six months (range: three days to six months) of their first panic attacks. In the single case where agoraphobia preceded the onset of panic attacks, the patient had had a longstanding history of separation anxiety since childhood. These data suggest that panic attacks and avoidance behaviors are tightly linked in time. Thus, when agoraphobia develops as a secondary complication, it is likely to occur within six months of the first panic attack.

Nineteen of 38 (50%) patients with panic disorder had a lifetime incidence of depression. While the symptoms themselves were quite severe as defined by our life charting criteria, it is noteworthy that only nine patients, or 24% of the entire sample, had ever had depressive episodes lasting greater than the two weeks duration required by DSM III for major depression. Patients that had depressions lasting greater than two weeks typically developed their first depressive episode either before (two patients) or within the first several weeks or months (less than 12 months) (seven patients) of the initial onset of panic attacks. In contrast, all patients with depressive symptoms lasting less than two weeks had their first depressions many months (greater than 12 months), and usually years, after their first panic attack. It should be underscored that with the exception of duration, there was no phenomenological difference in symptomatology between patients with short (less than two weeks) versus long (greater than two weeks) depressions. Depressions were endogenomorphic in character (anhedonia, depressed mood, terminal insomnia, excessive guilt, anorexia) with two notable exceptions: there was an absence of psychomotor retardation and suicidal ideation.

While the retrospective study of patients with panic disorder has a number of methodological flaws, this study represents one of the first attempts to obtain an accurate understanding of the longitudinal course of panic disorder using systematic life chart methodology. Our preliminary data using life charting techniques suggest that panic disorder is commonly associated with depressive symptomatology, although only 24% develop longstanding endogenomorphic symptoms. Psychomotor retardation and suicidal ideation is uncommon in panic disorder patients. The onset of panic attacks generally begin in adolescence or early adulthood and, if untreated, lead to an impaired lifestyle marked by pathological degrees of generalized anxiety and agoraphobia. Tricyclic antidepressants appear to have antipanic effects independent of the presence of concomitant depressive symptomatology. Careful attention to the longitudinal course of panic disorder may help to better elucidate the basic phenomenology and underlying mechanisms, and evaluation and production of treatment response.

3. Caffeine and Pathological Anxiety

Following the conclusions of a recent survey suggesting that patients with panic disorder may have an increased sensitivity to caffeine, our group (Drs. Boulenger, Uhde, Post and Bierer) decided to study the behavioral and biological effects of caffeine in panic patients and age- and sex-matched normal controls. Using double-blind, placebo-controlled conditions, we administered three doses of oral caffeine (240, 480, and 720 mg) to eight normal volunteers and two panic patients who were caffeine-free for at least one week prior to the

study. Several standard rating scales were administered at baseline and at 60 and 150 minutes after drug administration, while blood samples were obtained at baseline and at 30, 60, 90, and 150 minutes following drug administration.

In the combined group of normal controls and panic patients there was a dose-related increase in measures of state anxiety (ANOVA, $p < .04$), which was also apparent in the normal controls as a separate group. Thus, compared to placebo, normal subjects had increased ratings of anxiety following 240 mg (ANOVA, $p = 0.12$), 480 mg ($p = 0.06$), and 720 mg ($p = 0.01$) of caffeine. Two normal controls developed unequivocal panic attacks after receiving 720 mg of caffeine. There was no significant increase in MHPG at any dose up to 720 mg for the normal controls as a group. There was a significant negative correlation between baseline adenosine levels (assayed by N. Salem using HPLC) and the anxiogenic effects of caffeine. Plasma cortisol increased significantly compared to placebo following the 480 mg (ANOVA, $p = .05$) and 720 mg (ANOVA, $p = .003$) doses of caffeine. Of interest, the two normal controls who had panic attacks had a greater than five-fold increase in mean cortisol (peak concentration minus baseline value, $15.8 \mu\text{g/dl} \pm 1.9 \text{ SE.}$) after caffeine (720 mg) compared to the increase in cortisol ($2.8 \mu\text{g/dl} \pm 2.2$) ($p < .05$) in the six normals who did not experience panic attacks.

Preliminary results obtained in five patients with panic disorder suggest that panic attacks are more frequently induced by caffeine at lower doses than in normal controls and thus support our initial hypothesis.

4. Glucose and Lactate Metabolism

In collaboration with Dr. B.J. Vittone, seven of nine patients with panic disorder given a standard glucose tolerance test developed symptomatic hypoglycemia but not panic attacks. These preliminary findings indicate that the induction of hypoglycemia following a standard $1.5 \mu\text{g/kg}$ oral challenge with glucose does not "trigger" panic attacks in patients with panic disorder. Thus, hypoglycemia is an unlikely explanation for the "spontaneous" panic attacks that occur in patients with panic disorder or agoraphobia with panic attacks.

While none of our patients had panic attacks, a substantial and clinically significant proportion met two separate criteria for symptomatic hypoglycemia. Seven of nine (78%) patients had a hypoglycemia index above 1.0, while eight of nine (89%) patients had glucose nadirs below 60 mg/dl. Regarding the glucose nadir as an index of hypoglycemia, it is noteworthy that we employed the conservative measure of "plasma" glucose which has a 10%-15% greater concentration of glucose than the more frequently employed "blood" glucose levels reported in the hypoglycemia literature. Therefore, the mean "plasma" glucose nadir of 52 mg/dl in our study is roughly equivalent to a "blood" glucose range of 44-47 mg/dl. Although 17% of 285 asymptomatic, normal women have been reported to develop "blood" glucose nadirs below 59 mg/dl, it is notable that nearly all of our patients had nadirs within this hypoglycemic range. Enhanced insulin secretion following GTT has been associated with hypoglycemia to this degree. Whether our preliminary findings indicate disturbed insulin function in panic disorder is unclear but of interest since insulin-induced hypoglycemia activates neuropeptides implicated in the neurobiology of anxiety.

5. Clonidine: Probe of Noradrenergic Receptor Function

In an attempt to understand the dynamics of noradrenergic function in depression, our group (Drs. Uhde, Siever, Vittone, Jimerson, and Post) evaluated neuroendocrine, biochemical, and cardiovascular responses to the acute intravenous administration of the alpha-2 adrenergic agonist, clonidine 2 µg/kg, in depressed and panic patients and normal controls.

Previously we reported that growth hormone ($p < .05$) and plasma MHPG ($p < .05$) responses to clonidine were reduced in the depressed patients compared to the controls, all suggesting reduced responsiveness of alpha-2 adrenergic receptors in depression. Baseline levels of cortisol were elevated in the depressed patients compared to the controls (9.5 ± 5.5 mg/dl, $n = 16$, $p < .05$). Clonidine decreased cortisol to normal levels in the depressed patients but had little effect in the controls.

During the past year, our group (Drs. Uhde, Vittone and Post) has demonstrated that nondepressed panic patients have blunted growth hormone responses to clonidine similar to those found in patients with endogenomorphic depression. These results suggest that alterations in noradrenergic function may be similar in panic and major depressive disorders.

6. Clonidine as a Treatment for Anxiety

Alterations in noradrenergic function have been postulated in theories of anxiety, fear, and hyperarousal states. Redmond recently proposed a model for the study of anxiety based upon the noradrenergic nucleus locus coeruleus (LC). In animals, electrical or pharmacological activation of the LC produces fear-associated behaviors and increased norepinephrine (NE) turnover, whereas lesions or pharmacological inhibition produces decreased fear-associated behaviors, and decreased NE as well as its metabolite MHPG. In man, urinary, plasma, and CSF MHPG have been correlated with state anxiety.

Clonidine, an alpha-2 adrenergic agonist that inhibits LC activity, reverses the panic anxiety associated with opiate withdrawal and decreases plasma MHPG. These findings suggested to us that clonidine might have antianxiety effects in individuals with pathological degrees of anxiety. In order to explore this hypothesis, our collaborative group (Drs. Uhde, Siever, Vittone, Boulenger, Jimerson, and Post) has conducted two separate studies to assess the acute behavioral effects of clonidine in psychiatric patients. In the first study, clonidine (2 g/kg) was administered intravenously to 18 patients with major depressive disorders by Research Diagnostic Criteria (RDC) and 19 healthy normal volunteers. In the combined group of patients and normal controls, ratings of state anxiety, as measured by the Spielberger Anxiety Inventory, significantly decreased after clonidine (paired $t = 2.98$, $p < 0.006$, $n = 37$) but did not change after the placebo infusion. This antianxiety effect of clonidine was evident in the depressed patients as a separate group (paired $t = 2.56$, $p < 0.003$, $n = 18$) but not in normal controls. Although self-rated measures of drowsiness increased significantly ($p < .002$) in both groups, there was no correlation between the anxiolytic and sedative effects of clonidine. Consistent with a noradrenergic hypothesis of arousal and anxiety, subjects with the highest baseline levels of

plasma MHPG had the greatest improvement (drop in anxiety) after clonidine ($r = 0.41$, $p < 0.03$, $df = 28$).

In the second study, the same dose of intravenous clonidine was administered to 11 patients who met RDC criteria for panic and phobic disorders (agoraphobia subtype). Nine normal controls, seven of whom were included in the previous study, were used as a comparison group. Although the panic patients tended to have reductions in anxiety following placebo, clonidine's placebo-corrected effects remained highly significant ($p = .01$). Again, there was no correlation between sedation and degree of improvement. Several investigators have noted, however, that in clinical trials with clonidine tachyphylaxis may develop within three to four weeks, a time-course which parallels the loss of clonidine's inhibitory effects on locus coeruleus firing with chronic administration. It should be noted, however, that not all patients develop tolerance to clonidine's antianxiety effects. Furthermore, we have seen rather impressive improvement in some agoraphobic patients administered clonidine under double-blind, placebo-controlled conditions, including one patient who previously had been homebound. Thus, clonidine had acute antianxiety effects in both depressed and panic-anxious patients. Although the normal controls as a group showed no change in anxiety after clonidine, those normal volunteers with the highest ratings of baseline anxiety in our study did experience the greatest decrement in anxiety. Together, these findings are consistent with an emerging body of data suggesting that clonidine has anxiolytic properties. Although clonidine has indirect effects on other neurotransmitter systems, its antianxiety effects are most likely mediated by dampening noradrenergic overactivity.

7. Clonidine and Plasma Melatonin

The effect of clonidine on plasma melatonin during sleep has been studied in collaboration with Drs. A. Lewy and L.J. Siever. Clonidine $2 \mu\text{g}/\text{kg}$ i.v. produced at least a 50% reduction in plasma melatonin in all normal controls. This preliminary finding is noteworthy and provides a unique methodology by which noradrenergic responsivity in anxious patients may be assessed.

8. Yohimbine Challenge

Yohimbine is a relatively selective alpha-2 adrenergic antagonist which acts at the presynaptic level to enhance, rather than reduce, the neuronal release of norepinephrine in the central nervous system. In animals, yohimbine may increase behaviors related to fear and has some of its effects antagonized by diazepam. In man, previous investigations found that yohimbine induced anxiety and autonomic symptoms such as tachycardia and sweating in various groups of psychiatric patients when given at high doses. However, yohimbine given orally at lower doses has not been found to be associated with anxiety in normal controls. In collaboration with Drs. B.J. Vittone, J.-P. Boulenger, and R.M. Post, a low-dose yohimbine (20 mg p.o.) oral challenge paradigm has been initiated to test the hypothesis of an increased noradrenergic sensitivity in patients with panic disorder compared to normal controls and affectively ill patients without panic attacks.

Nine of the first 11 panic patients studied to date developed profound anxiety, including several patients who had panic attacks after yohimbine,

whereas none had anxiety to this degree or panic attacks while receiving placebo ($p = .003$). There was a nonsignificant trend for the five normal volunteers to experience an increased sense of well-being, rather than increased anxiety, after yohimbine. This apparent increased vulnerability to yohimbine probably reflects an abnormality in noradrenergic function or receptor sensitivity in patients with panic disorder rather than a conditioned response to internal signals previously associated with distress.

9. Anxiety and Pain

The relation between anxiety and pain has been extensively explored over the past thirty years. Several studies have suggested that medical and surgical patients with high anxiety levels seem to experience greater pain. Other studies have shown that both experimentally-induced anxiety in normal volunteers and higher levels of trait anxiety in psychiatric patients are associated with greater sensitivity to pain. However, these earlier studies were based largely on pain measurement procedures that confounded sensation and response variables, making it difficult to determine whether anxiety affects pain sensation or just the tendency to report pain.

Three recent studies in normal subjects have utilized pain rating procedures based on signal detection analyses in order to permit separate identification of the effects of anxiety on subjects' response bias (the level of stimulus intensity that best separates two sensory judgements and is presumably a function of cognitive and motivational factors) and on subjects' ability to discriminate varying intensities of stimuli (how little overlap there is between sensory judgement which is presumably a function of sensory-perceptual variables). Although signal detection analysis may not achieve such an absolute separation of cognitive and sensory variables, several studies have shown that only the discriminability measure is related to analgesia (pain insensitivity) in humans. All three studies failed to demonstrate that anxiety increased pain sensitivity. In fact, we previously reported that increased anxiety in normals decreased pain sensitivity.

We conducted the first study to explore the relationship between pain and anxiety in patients suffering from primary anxiety disorders identified by modern diagnostic criteria. In collaboration with Drs. P. Roy-Byrne and M. Buchsbaum, an index of pain insensitivity was obtained for 18 panic patients and age- and sex-matched normal volunteers. Subjects received three shocks at each milliamperage (MA) increment from 1 to 31 for a total of 93 randomly presented shocks. Subjects judged each shock as noticeable, distinct, unpleasant, or very unpleasant. The index of pain insensitivity is derived from the subjects' ability to distinguish between distinct and unpleasant sensations. Increases in this measure have been associated with morphine and aspirin analgesia and decreases with naltrexone-induced hyperalgesia in schizophrenics. In addition, the milliamperage level that best separates the "distinct" and "unpleasant" judgements was computed (i.e., the stimulus intensity for which the least overlap between judgements occurred) and termed "response criterion". It is a measure of the patients' propensity to call stimuli painful. The number of responses out of the total of 93 judged "unpleasant" or "very unpleasant" were counted and termed "pain counts". Also, the mean milliamperage intensity for stimuli termed

"unpleasant" or "very unpleasant" was also computed as a rough approximation of "pain threshold".

There was no significant difference between the patient and control group in the insensitivity index ($t = -1.26$, NS), response criterion ($t = 1.34$, NS), number of pain counts ($t = -1.07$, NS), or mean intensity of unpleasant or painful stimuli ($t = -0.33$, NS). There also was no relationship between anxiety and either pain sensitivity ($r = -.31$, NS), response criterion ($r = .22$, NS), number of pain counts ($r = -.20$, NS), or mean intensity of unpleasant or painful stimuli ($r = -.04$, NS). The number of panic attacks in the month prior to testing was also unrelated to any of the above four criteria.

Comparison of the slopes of the best fit lines to the bivariate plot of anxiety and insensitivity index in normals ($m = 0.65$) and patients ($m = -0.74$) revealed that the slopes were significantly different ($t = -2.93$, $p < .01$). Comparison of the correlation coefficients between anxiety and insensitivity index in normals ($r = .62$) and patients ($r = -.31$) also yielded a significant difference ($z = 2.2$, $p < .03$).

Thus, in this study, using both threshold pain measures and signal detection measures, we failed to demonstrate any difference in pain sensitivity between patients with panic disorder and normal controls. Despite their similarity to normals on all measures of pain sensitivity, the fact that they do not demonstrate the same type of correlation between levels of anxiety and pain is provocative and deserves further exploration.

11. Anxiety and Sleep Architecture

Insomnia is commonly believed to result from anxiety or other states of increased autonomic arousal. In accordance with this hypothesis many clinicians have employed relaxation techniques, biofeedback, systematic desensitization, and antianxiety pharmacotherapy in the treatment of insomnia. Although anxiety is a frequent concomitant of insomnia, no laboratory has investigated either the prevalence of insomnia or the sleep architecture of patients who meet Research Diagnostic Criteria (RDC) for the panic and phobic disorders. Furthermore, a comparison of sleep between panic anxious and depressed patients, as well as normal controls, is indicated since both panic anxious and depressed patients respond to tricyclic and MAO inhibitor drugs. In collaboration with Drs. P. Roy-Byrne, J.C. Gillin, and W. Mendelson, we are investigating both the frequency of complaints of insomnia and the sleep architecture of nine patients who meet RDC for panic and phobic disorders.

All nine panic disorder patients reported sleep disturbances characterized by difficulty falling asleep and multiple nocturnal awakenings during the sleep study. All patients attributed their disturbed sleep to fears of impending panic attacks and a perceived inability to escape from the hospital under such circumstances. One patient actually had a panic attack shortly after awakening at 2:00 am and finding herself in an unfamiliar environment. Unfortunately, she did not inform the nursing staff until the following day so that her sleep architecture immediately prior to the attack could not be identified.

Analysis of sleep EEG's were only partially consistent with the patients' reports of restless, broken sleep. Although patients had significantly increased movement time, the mean (\pm SD) total time awake was actually nonsignificantly decreased in the panic patients (16.8 ± 23.0) compared to the controls (30.7 ± 28.6). The panic disorder patients did have both a significantly shorter REM latency ($p < .05$) and a decreased REM density ($p < .05$). The following sleep variables (mean \pm SD) were not significantly different in the panic anxious patients and controls respectively: total time asleep; sleep latency; total non-REM time; REM time; eye movements; time in state 1, stage 2, stage 3, stage 4; delta sleep; % of delta sleep; % of REM sleep; number of REM periods; and sleep efficiency (total sleep/total recording period). Tabulating the mean length of each consecutive REM period demonstrated no difference between the groups (2-way ANOVA, NS), although both panic patients and controls had similar and significant ($p = .02$) increases in REM length with each successful REM period.

Total sleep ($r = -.81$, $p = .008$) and REM % ($r = -.80$, $p = .009$) related inversely to measures of "global" anxiety but were not correlated with depression. Frequency of panic attacks showed a negative correlation with delta sleep ($r = -.73$, $p = .04$). Of interest, REM latency showed a positive correlation with frequency of panic attacks ($p = .05$) and severity of depression ($p = .06$).

Although the panic patients had shorter REM latency, it was nearly twice as long as that typically reported in patients with endogenous depression. Thus, REM latency does not appear to be similar in these two groups.

A major finding in the present study was the increased movement time, a measure which may be linked to the patients' subjective reports of disturbed sleep. This finding is also consistent with a condition of chronic hyperarousal in this population. Although this finding clearly requires confirmation by more precise methods, a pattern of increased psychomotor activity throughout the sleep-wake cycle would be noteworthy in relation to abnormal biological rhythms that have been reported in patients with affective illness.

12. Alpha-Adrenergic Receptors

In collaboration with Dr. M. Kafka, alpha-adrenergic function was measured in the platelets of patients with panic disorder and in age- and sex-matched normal controls. ^3H -dihydroergocryptine ($^3\text{HDHE}$) binding was increased, whereas prostaglandin E_1 (PGE_1)-stimulated cyclic AMP (cAMP) production was decreased in patients compared to controls. The percent inhibition by norepinephrine of PGE_1 -stimulated cAMP was lower in patients than controls. Similar alterations in alpha-adrenergic function have been found in patients with major depressive illness. These data suggest that similar alterations in noradrenergic function may occur in the panic and affective disorders.

D. Proposed Course of Project

During the past year, the Unit on Anxiety and Affective Disorders has established a colony of "normal" and "nervous" pure-bred pointer dogs. These dogs offer the advantage of investigating both "normal" behavior and "spontaneously-occurring" (rather than laboratory-conditioned) fear behaviors. The "nervous" line may be particularly useful in the study of several behaviors and

characteristics relevant to human psychopathology, including genetically-transmitted inheritance with phenotypic expression of "nervous" behaviors at 8-12 months of age. This delayed manifestation of pathology in dogs parallels in a similar, temporal fashion, the emergence of agoraphobia in humans during adolescence and early adulthood.

Human research conducted by the Unit on Anxiety and Affective Disorders has demonstrated a blunting of the clonidine-induced growth hormone (GH) response in affectively ill patients compared to age- and sex-matched controls. These findings, which have been replicated by four independent research groups, may suggest decreased postsynaptic noradrenergic function in endogenous depression. Increasing evidence also suggests a heterogeneity in anxiety and depressive syndromes. Thus, the blunted (GH) response to clonidine in patients with panic disorder (without depression) suggests that these syndromes have in common similar disturbances in noradrenergic function.

We plan to continue the yohimbine challenge study to further assess noradrenergic function in patients with pathological anxiety and depression. The behavioral and biochemical effects of alprazolam and carbamazepine on yohimbine-induced anxiety will be investigated in panic patients. Single-dose diazepam, a standard antianxiety agent, will also be given to patients and controls to investigate the relationships among anxiety, psychophysical pain, and various peripheral correlates.

We also intend to continue our challenge studies with caffeine. Further delineation of the clinical response to caffeine is indicated because caffeine consumption is correlated with symptoms of generalized anxiety in patients with panic attacks, but not in normal volunteers. Caffeine derivatives also activate noradrenergic activity in animals when iontophoretically applied to the LC. Furthermore, caffeine has been shown by others to antagonize the biochemical and pharmacological effects of benzodiazepines in humans. We intend to extend these preliminary studies by investigating the behavioral and biochemical effects of alprazolam on caffeine-induced anxiety. Since caffeine has been reported to induce arrhythmias in patients with mitral valve prolapse (MVP), we will investigate the relationship among caffeine sensitivity, MVP, anxiety, and arrhythmias in patients with primary anxiety disorders.

Challenges with TRH and CRF will be initiated as well to further characterize the hypothalamic-pituitary-adrenal axis in anxious patients.

Studies of clinical efficacy of alprazolam, clonidine, carbamazepine, imipramine, and propranolol in panic and phobic patients will be continued. Our preliminary research with clonidine in depressed and anxious patients and normal volunteers is encouraging and suggests that clonidine might be especially useful in patients who experience context-dependent anxiety (e.g., anticipatory anxiety) as well as some patients with "spontaneous" panic attacks. Studies with verapamil, a calcium channel blocker, will also be initiated. These clinical trials, in conjunction with concomitant measurements of the neurotransmitter effects, should enhance our understanding of alterations in neurotransmitter pathways associated with pathological anxiety and its amelioration with appropriate psychopharmacotherapies.

E. Significance to Biomedical Research and the Program of the Institute

Several epidemiological surveys have suggested that pathological degrees of anxiety may adversely influence a large segment of our population. Agoraphobia, an anxiety syndrome associated with "spontaneous" panic attacks, results each year in the impairment of individuals previously well-functioning and productive. Pathological anxiety has been recently found to be the second most prevalent mental health problem in this country. Approximately 25 million individuals suffer from phobias and related fears and pathological anxiety. The role of anxiety and stress in coronary heart disease and other medical illnesses has been suggested by a number of studies. Moreover, emerging epidemiological and familial data suggest that a subgroup of patients with major depressive illness plus panic attacks may represent an important and distinct subtype of major affective illness. We intend to investigate biological correlates in the plasma and cerebrospinal fluid of this subtype, who may be a greater risk for alcoholism and suicide, compared to patients with major depressive illness without panic attacks. An improved understanding of the clinical and biological aspects of both normal and pathological anxiety is thus critically needed. It is hoped that the developing battery of clinical and biological tests in patients with anxiety and related mood disorders will ultimately provide a clinical and biological profile of these illnesses and lead to more refined subcategorizations, as well as to more selective and efficacious treatment approaches.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00452-09 BP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neuroendocrine Studies of Major Psychiatric Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Philip W. Gold, M.D.

COOPERATING UNITS (if any)

Tufts Univ., Boston; Developmental Endocrinology, NICHD; U. of Chicago, Experimental Ther. Branch, NICHD, Surgical Neuroendocrinol. Branch, NINCDS, NIH.

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Unit on Neuroendocrinology

INSTITUTE AND LOCATION

NIMH, Bethesda, MD 20205

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

5.4

3.0

2.4

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Current studies concentrate on elucidating the physiology and clinical relevance of peptide neurohormones (e.g., corticotropin releasing factor) which influence both central nervous system function and pituitary-adrenal regulation. In volunteers, continuous infusion of ovine CRF (oCRF) produces moderate hypercortisolism associated with a phase advance in the circadian rhythms of ACTH and cortisol, analogous to that seen naturalistically in depression. Continuous ACTH infusion to volunteers produces cortisol responses similar to those seen in Cushing's disease. Analysis of ACTH and cortisol responses to oCRF in depression and Cushing's disease supports the hypothesis of excessive endogenous CRF secretion in depression and autonomous corticotropin cell ACTH overproduction in Cushing's disease. The CRF test can distinguish depression from even mild Cushing's disease with 95% accuracy when the ACTH/cortisol ratios obtained during CRF testing are compared. The CRF stimulation test is also an aid in the differential diagnosis of adrenal insufficiency and in distinguishing Cushing's disease from ectopic ACTH secretion. The responses to CRF testing in patients with anorexia nervosa and primary anxiety disorder suggest that the pathophysiology of hypercortisolism in the disorders is similar to that of depression. Comparisons of the pharmacokinetics and biological effects of oCRF and human CRF (hCRF) in primates and volunteers show that the two peptides are equally potent in releasing ACTH; however, the metabolic clearance rate of hCRF is ten times faster than that of oCRF. hCRF pulses given to volunteers and patients with hypothalamic CRF deficiency produce ACTH pulses whose duration and amplitude mimic endogenous secretory episodes. In the sheep, stress (i.e., insulin-induced hypoglycemia or benzodiazepine superantagonist administration) causes a significant rise in ACTH in association with a rise in CSF CRF.

COLLABORATORS:

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- Dr. J. Rock, Staff Physician, NINCDS
- Dr. P. Ninan, Staff Physician, NINCDS
- Dr. H. Schulte, Visiting Fellow, NICHD

Project Description

Objectives: Several aspects of the symptom complex of the functional psychoses, particularly affective illness and anorexia nervosa, suggest hypothalamic dysfunction. For instance, patients with depression or mania often show disturbances in sleep, altered energy levels, changes in appetite and libido, diurnal variation in symptoms, alterations in the consolidation of memory traces, and changes in reproductive function such as amenorrhea. Anorexics show not only profound alterations in appetitive behavior, but also marked functional changes in the hypothalamic-pituitary axis, including abnormalities in gonadotropin, thyrotropin, growth hormone and vasopressin secretion. Interest in the hypothalamic-pituitary axis has also been stimulated by recent findings that the monoaminergic neurotransmitters modulate the synthesis and release of a number of hypothalamic peptides and pituitary hormones; thus, examination of pituitary hormones in plasma can shed light on the functional activity of biogenic amine systems. Moreover, the hypothalamic hormones themselves have been shown to be widely distributed in brain, exert specific receptor-mediated biological actions, and influence the functional activity of brain neurotransmitter systems. Several hypothalamic hormones have also been shown to have profound effects on complex behaviors and cognition.

In our clinical studies, several neuroendocrine strategies have been routinely utilized: 1) direct measurement in the cerebrospinal fluid (CSF) and in the plasma of behaviorally active peptides during the basal state and/or following stimulation according to verified stimulation paradigms; 2) administration of hypothalamic releasing factors to test responses of the hypothalamic-pituitary axis and to elucidate patterns of monoaminergic disturbance and neuroendocrine dysfunction; 3) effects of psychoactive drugs on hypothalamic-pituitary axis function and on the levels of behaviorally active peptides; 4) assessment of the temporal organization of neuroendocrine function; 5) assessment of the relationship between neuroendocrine function and sleep.

For purposes of comparison and possible differential diagnosis, normal subjects, patients with affective illness, schizophrenia, anorexia nervosa, and Cushing's disease are studied. Our group is also actively involved in studying the neurobiology of several neuroendocrine diseases, particularly Cushing's syndromes, as well as Kallman's disease, and in developing clinical means for the differential diagnosis of Cushing's disease. In addition to these clinical studies, we have studied neurohormonal function in stalk-sectioned and intact subhuman primates to examine dose-response relationships, pharmacokinetics, and the physiology of peptide secretion into the plasma and CSF spaces. Animal models have also been established in the sheep and other species. This work is supported by our own laboratory which has developed assays for a wide variety of peptide neurohormones.

Methods Employed1. Metabolic Clearance Studies

Methods consist of: 1) radioactive labelling of peptides such as CRF; 2) measurement of CRF and other peptides by radioimmunoassay and

estimation of its metabolic clearance rate by pulse injection and continuous infusion studies; 3) radioimmunoassay - radioimmunoassays have been developed to measure ovine and human CRF, ACTH, beta-endorphin, arginine vasopressin, and oxytocin; 4) dose responses have been performed to assess the effect of ovine and human CRF on plasma ACTH, beta-endorphin and adrenal steroids in both non-human primates and man; 5) CRF receptor assay has been used to examine CNS and pituitary CRF receptors, and in peripheral tissues, in an effort to explain the peripheral hemodynamic effects of CRF in primates; 6) a primate model with a Swan-Ganz catheter, an aorta catheter, and various electromagnetic flow probes around major vessels, have been developed to examine the peripheral cardiovascular effects of CRF; 7) a sheep model has been developed with indwelling intracerebroventricular cannulae to assess the circadian rhythm of CRF, vasopressin, beta-endorphin, ACTH, and the cerebrospinal fluid; simultaneous plasma samples are also obtained in this paradigm; 8) a primate model of depression, termed "the marmoset wasting syndromes," has been developed requiring a placement of intracerebroventricular cannulae, indwelling intravenous lines, and establishment of validated criteria for assessment of clinical status.

Studies of Behaviorally Active CNS Peptides

1. Corticotropin Releasing Factor (CRF)

A major area of work this year has been on basic and clinical studies of corticotropin releasing factor (CRF). Since this 41 amino acid peptide was first isolated from ovine hypothalami, our initial studies were conducted with this ligand (oCRF). Recently, another 41 amino acid corticotropin releasing factor, which shows 87% structural homology with oCRF, has been isolated from rat hypothalami. This peptide is thought to be identical with human CRF; thus, we have begun extensive studies with this ligand (r/h CRF) to compare its effects to those of oCRF.

The sequencing of oCRF and r/h CRF provides the most direct opportunity so far available to study central control of the hypothalamic-pituitary-adrenal (HPA) axis in man and coincides with an increasing interest in the regulation of the HPA axis in patients with affective disorder and of other psychiatric subgroups shown to manifest sustained hypercortisolism during some phase of their illness. CRF is also of interest to us since it has been shown that it is the principal control signal for the cleavage of pro-opiomelanocortin, which not only contains the sequence of ACTH but of beta-endorphin as well. Thus, CRF may play an important role with respect to endogenous opiate activity. Moreover, CRF, like many other hypothalamic peptides, is synthesized and distributed in disparate extra-hypothalamic sites, and may play a role in orchestrating a variety of physiological and behavioral events classically associated with response to stress. For instance, intracerebroventricular administration to the rat leads not only to the activation of the HPA, but also to stimulation of the sympathetic nervous system, and to several behavioral changes characteristic of the stress response, including decreased feeding and sexual behavior as well as assumption of a freeze posture in a foreign environment and increased exploration in familiar surroundings. In addition, in collaboration with Dr. R. Post, it has been shown that CRF given ICV to the

rat causes a marked increase in hostility and induces limbic seizures which show cross-sensitization with electrically kindled seizures.

Given CRF's significant role in HPA regulation and its intriguing effects on CNS function, we embarked on a series of clinical studies with oCRF and r/h CRF in rat, sheep, and primate models, and in normal volunteers and patients with a wide variety of psychiatric and nonpsychiatric disorders characterized by abnormalities in HPA function. Some of the questions we asked are as follows: (1) Can CRF help to determine whether the hypercortisolism in depression reflects an alteration in the set point for feedback inhibition of cortisol on ACTH secretion at a pituitary locus versus the possibility of an alteration in the secretion of endogenous CRF itself? (2) Can CRF help in determining whether depression or Cushing's disease lie on a common pathophysiological continuum, or whether they represent distinct abnormalities of the HPA axis? (3) Can CRF help in establishing the differential diagnosis of disturbances in HPA function which can be difficult to distinguish from one another, such as depression from Cushing's disease, ectopic ACTH production from pituitary Cushing's disease or hypothalamic pituitary-adrenal insufficiency? (4) What factors regulate CRF secretion into the hypophyseal portal system or into centrally directed pathways which convey the peptide to the cerebrospinal fluid?

Studies of CRF Secretion and Function in Animal Models

Our initial studies in animal species were in stalk-sectioned and intact primates to determine the dose-response relationship and pharmacokinetics of the first of the available CRF ligands (i.e., oCRF). It was shown, in collaboration with Dr. Schulte, that the lowest maximal stimulatory cortisol secretion was 1 ug/kg. It provoked a prolonged ACTH and cortisol response; this proved to be secondary to the prolonged half-life demonstrated for oCRF in plasma, which was greater than 70 minutes for the long component. This work led to one of the first applications of the safe, clinically useful paradigm in human subjects. Shortly after the sequencing of r/h CRF, in collaboration with Dr. Schurmeyer, we determined the biological effects and the pharmacokinetics of this peptide in the rhesus monkey. r/h CRF proved to be equally potent to oCRF (i.e., lowest stimulatory dose of cortisol, 0.5 ug/kg, lowest maximal stimulatory dose for p cortisol response, 1 ug/kg). However, the duration of r/h CRF's effect on the pituitary-adrenal axis and its plasma half-life proved to be much shorter than for oCRF, and the metabolic clearance rate for r/h CRF was more than ten times faster than for oCRF. We have also explored the effects of continuous administration of oCRF to primates for as long as two weeks, demonstrating that such administration did not result in either sensitization or profound desensitization of the corticotroph.

In addition to the foregoing primate work focused on the physiology of oCRF and r/h CRF, we were also interested in following up on the work indicating that centrally administered CRF could influence many of the behavioral and physiological processes classically associated with stress. For instance, in collaboration with Dr. Oldfield, it was demonstrated that CRF is actively cleared from the cerebrospinal fluid, suggesting a possible role for this pathway in mediating some of CRF's putative central effects. We also showed, in collaboration with Dr. Rock, that ICV CRF increased CSF ACTH

concentrations. In addition, in collaboration with Dr. Ninan, it was shown that administration of a benzodiazepine antagonist, BCCE, resulted in a profound rise in plasma ACTH and a concomitant significant display of manifest anxiety. This response may have been mediated by CRF, as suggested by data obtained from centrally cannulated sheep, which will be reviewed below. Consistent with the possible role of CRF in stress-mediated phenomena, in collaboration with Dr. Udelsman, it has been shown in the rhesus monkey that r/h CRF provokes a dramatic and prolonged fall in both peripheral vascular resistance and mean systolic pressure. This finding suggests that r/h CRF may function as a paracrine hormone modulating local blood vessel tone, possibly directing blood flow during stress and injury.

An additional major preclinical undertaking has been the elucidation of the regulation of the secretion of ACTH and oCRF secretion into the CSF and plasma of the sheep. Simultaneous blood and CSF was obtained in the indwelling intravenous catheters and an Ommaya-type reservoir. Basal plasma and CSF cortisol, ACTH, and CRF levels were obtained with repeated and prolonged sampling. It was noted that there was a significant negative correlation between basal plasma cortisol and CSF CRF, and between basal plasma ACTH and CRF, suggesting a physiologic link between central CRF secretion and pituitary-adrenal functional activity. CSF ACTH did not correlate with any measure of pituitary-adrenal function. Pharmacologic or physiologically-induced stress produced concomitant increases in plasma cortisol and ACTH and in CSF CRF secretion. For instance, BCCE, the benzodiazepine antagonist, produced a dramatic rise in plasma ACTH associated with a clear but subtle increase in CSF CRF. This finding suggests the possibility that the GABA-ergic system tonically inhibits the CRF neuron, and that the anxiety associated with this disinhibition could relate to the actions of central CRF. Work is currently in progress to see if BCCE-induced CRF secretion is associated with intense anxiety in the setting of pretreatment by ICV administration of a specific CRF antagonist.

In collaboration with Dr. R. Post, we have also studied the effects of another benzodiazepine antagonist, RO4864. This substance interacts with the so-called "peripheral" benzodiazepine site in brain which is also thought to be a potential locus of carbamazepine action. The effects of RO4864 were similar to those of BCCE (i.e., activation of plasma ACTH in association with a modest rise in CSF CRF). Another stressful stimulus which was applied was hypoglycemia, which does indeed raise plasma ACTH in association with a rise in CSF CRF. We also studied the effects of procaine administration on plasma ACTH and CSF CRF secretion, since we have shown, in collaboration with Drs. R. Post and C. Kellner, that procaine causes a clear rise in plasma ACTH, either in the presence or absence of associated mood changes secondary to this agent. Since procaine is thought to cause limbic seizures and has been shown to cause temporal lobe activation in humans, we wanted to see if procaine influences CRF secretion, as CRF has been shown to promote limbic seizure activity. Preliminary data shows that procaine, like the other agents, causes a significant rise in plasma ACTH in association with a small but palpable rise in CSF CRF.

Studies with oCRF and r/h CRF in Normal Volunteers

Studies oCRF and r/h CRF in normal volunteers, in collaboration with Dr. Schulte and Dr. Shurmeyer, replicate the findings of the dose-response and pharmacokinetic studies with these peptides in primates. The demonstration that oCRF and r/h CRF differ markedly in their pharmacokinetic properties has implications for the potential clinical application of these peptides. For instance, the prolonged action of oCRF could make it more suitable for diagnostic studies, while the brief action of r/h CRF could make it more suitable for studies of the naturalistic pulse frequency of ACTH secretion. In support of this latter possibility is the recent work with Dr. Shurmeyer, which shows that a single 1 ug/kg bolus of r/h CRF provokes an ACTH response that is virtually identical in both its amplitude and duration to naturalistic ACTH pulses obtained in normal volunteers with very frequent blood sampling. An additional study with Dr. P. Avgerinos strongly implicates CRF as a physiologically important modulator of pulsatile ACTH secretion. This study, conducted in patient volunteers with documented CRF-responsive adrenal insufficiency (i.e., hypothalamic-adrenal insufficiency) showed that administration of nine 1 ug/kg IV bolus injections of CRF over a 24-hour period (at the hours corresponding to expected pulses of ACTH) produced a pattern of ACTH and cortisol secretion which was very similar to that which we obtained in studies of normal volunteers. Moreover, the 24-hour urinary-free cortisol excretion in these patients was almost identical to that seen in a group of age-matched controls.

In collaboration with Dr. Schulte, the effects of continuous administration of oCRF on ACTH and cortisol secretion in volunteer subjects were determined. It was noted that neither sensitization nor desensitization of the corticotroph occurred, but rather a moderate activation of the pituitary-adrenal axis (i.e., about a 50% increase in the mean amplitude of the 24-hour cortisol secretion). Moreover, the circadian rhythms of cortisol and ACTH were preserved (though phase advanced) despite constant pharmacological plasma levels of oCRF. Of possible clinical significance is the finding that the pattern of the hypercortisolism induced by continuous CRF infusion is similar to that seen in most forms of depression ("pseudo-Cushing's disease"). On the other hand, it was demonstrated that the hypercortisolism of Cushing's disease was much more closely reproduced by a continuous infusion of ACTH rather than oCRF. Thus, this study in volunteers indirectly suggests that the pathophysiology of hypercortisolism in depression and Cushing's disease is distinct.

Clinical Applications of CRF in Non-Psychiatric and Psychiatric Disorders Characterized by Abnormalities of HPA Function

More direct explorations of the potential clinical relevance of CRF have been conducted in patients with a variety of disorders of the hypothalamic-pituitary-adrenal axis. One important observation is that CRF testing seems a potential aid in establishing the differential diagnosis between ectopic ACTH and Cushing's disease. Hence, patients with ectopic ACTH secretion generally failed to respond to CRF, while patients with Cushing's disease show a markedly exaggerated ACTH response to CRF.

CRF testing also seems of potential value in establishing the differential diagnosis of adrenal insufficiency. As expected, patients with primary adrenal insufficiency show exaggerated ACTH responses to CRF, and those with pituitary lesions show absent or minimal ACTH responsiveness. A third group was identified, however, which is CRF-responsive, showing a delayed or prolonged ACTH response to CRF. This group presumably manifests a component of hypothalamic CRF deficiency. Hence, CRF seems helpful in distinguishing between different forms of secondary adrenal insufficiency.

CRF testing in patients with Nelson's syndrome reveals that these tumors are exclusively CRF-sensitive, and that the response is highly correlated to tumor size. As expected, continuous CRF administration did not desensitize these tumors' response to CRF since ACTH levels were not significantly affected by this intervention. Although it was not established whether CRF response to Nelson's tumors are CRF-dependent, it is possible that these lesions may respond therapeutically to recently developed CRF antagonists.

We have conducted CRF testing in a total of 36 drug-free patients with primary affective disorder, of whom 18 were depressed, 8 were manic, and 10 improved. One purpose for undertaking this test was to contrast the responses seen in depression with those obtained in Cushing's disease. This is of clinical significance since the depressed phase of primary affective disorder is frequently associated with sustained hypercortisolism (at times of sufficient magnitude to be termed a "pseudo-Cushing's state") while subjects with Cushing's disease show signs of clinical depression. Although the etiology of the depression and hypercortisolism seen in affective illness and Cushing's disease is unknown, the overlap in the biochemical and clinical manifestations of these illnesses has prompted some to suggest that they share common pathophysiological features.

Compared to control subjects, depressed patients showed a significant increase in basal plasma cortisol which was associated with significant reductions in the net ACTH and cortisol responses to CRF. Moreover, there was a trend for significant negative correlation between basal cortisol and the net ACTH response to CRF in depressed patients. Thus, the net ACTH response to CRF tended to be most attenuated in the severely hypercortisolemic (pseudo-Cushing's) group of depressed patients. In contrast to depressed patients, the manic and improved patients showed normal basal cortisol levels and normal ACTH and cortisol responses to CRF. The negative correlation between basal cortisol and the net ACTH response to CRF persisted when these parameters were examined in the overall group of depressed, manic, and improved subjects.

The clearance of CRF was similar in normal subjects, each of the affective illness subgroups and, indeed, in all of the subgroups of patients tested.

An additional finding of the study was that in depressed patients the ACTH and cortisol responses to CRF were both significantly reduced but the magnitude of the reduction was substantially greater for ACTH than for cortisol. Thus, compared to controls, the depressed patients showed a proportionally greater cortisol response to the amount of ACTH released during CRF stimulation; this

was reflected in the finding that the ratio of the ACTH/cortisol response during CRF infusion was significantly less in depressed patients than in normal subjects.

It is clear that the responses to CRF in depressed patients are markedly different from those seen in patients with Cushing's disease. Indeed, this data indicates that the pathophysiologic loci of the hypercortisolism in depression and Cushing's disease are distinct. With respect to the pituitary component of the HPA axis, the blunted ACTH and cortisol responses to exogenous CRF in depression indicate normality of corticotroph cell function (i.e., the negative feedback effects of cortisol at the pituitary are intact, effectively serving as a brake on ACTH secretion during exogenous administration). This formulation is supported by the negative correlation noted between basal cortisol concentrations and the net ACTH response to CRF in depression. On the other hand, the corticotroph cell in patients with Cushing's disease is grossly unresponsive to the negative feedback effects of cortisol, since these patients show a remarkably exaggerated ACTH response to exogenous CRF despite their highly elevated basal cortisol levels.

Differences in pituitary corticotroph cell function between depressed and Cushing's patients are also accompanied by apparent differences in hypothalamic CRF neuron function. Hence, in depressive illness, where there is normal corticotroph cell function, we postulate the presence of a hypothalamic abnormality which results in the hypersecretion of endogenous CRF. This postulate is supported by the finding, noted above, that a continuous infusion of CRF in higher doses than for normal volunteers produces a pattern of cortisol secretion similar to that seen in depression. On the other hand, the ACTH responses to CRF in patients with Cushing's disease studied one week after successful microadrenectomy suggest the presence of a suppressed rather than hyperactive hypothalamic neuron. Specifically, these patients, who typically manifest undetectable plasma ACTH levels in the weeks following surgery, show normal or nearly normal plasma ACTH responses to exogenous CRF. Thus, one can surmise that their post-operative course would have been uncomplicated by adrenal insufficiency if only their own feedback responsive CRF neurons had not been suppressed by long-standing exposure to highly elevated levels of plasma cortisol.

Another difference in the pattern of response between depressed patients and patients with Cushing's disease is in the net ACTH/cortisol ratio obtained during CRF stimulation. The finding of a proportionally greater cortisol response to the ACTH released during CRF stimulation in depression (reflected in the low net ACTH/cortisol ratio) is compatible with development of functional and anatomic hypertrophy of the adrenal cortex known to occur during chronic hyperstimulation with ACTH or during chronic stress.

Although both the depressed and Cushing's disease patients are hypercortisolemic, basal plasma ACTH levels are in the normal range in depression. This normal basal plasma ACTH most likely reflects the fact that our chronically depressed patients show an exaggerated cortisol response to ACTH in the setting of a corticotroph cell which responds both physiologically to negative feedback from below and is excessively driven by exogenous CRF from

above. Hence, the corticotroph, restrained by negative feedback to secrete at a rate which produces plasma ACTH levels in the normal range is driven sufficiently by CRF to promote excessive cortisol secretion by hyperplastic adrenals. This suggests that the pattern of responsivity to CRF in depression will vary according to the duration and severity of the hypercortisolism.

We have also explored the hypercortisolism of other major psychiatric disorders such as anorexia nervosa, primary anxiety disorder, and schizophrenia. Somewhat surprisingly, underweight patients with anorexia nervosa show a pattern similar to that seen in depression; when these patients are studied one month after recovery of normal weight, they still show blunted ACTH response to CRF, but the cortisol responses have normalized. This suggests that the altered set point for CRF secretion at a hypothalamic locus in anorexia nervosa normalizes with the recovery of normal weight. Persistence of blunted ACTH responses probably represents the fact that the adrenals remain functionally hypertrophied for several weeks after restoration of the chronic hypercortisolism to normal. Patients with primary anxiety disorder, studied in collaboration with Drs. P. Roy-Byrne, T. Uhde, and R. Post, also show blunted ACTH response to CRF in association with hypercortisolism. This is the first study which really establishes that the axis in these patients appears to be hyperactive. The homogeneity of the responses to CRF in these hypercortisolemic subgroups of depressed patients may not represent a non-specific stress situation in these subjects; indeed, this would not appear to be the case since normals under chronic stress show a gradual normalization of cortisol hypersecretion after only a few days, in contrast to the prolonged and sustained hypercortisolism seen in psychiatric patients. Rather, this finding may suggest that hypersecretion of CRF may be a common finding in a variety of psychiatric subgroups which, although disparate in many of their clinical manifestations, share the feature that depression is often a clinical concomitant of each of them. We have proposed a model, published in a special article in the American Journal of Psychiatry to account for the potential role of CRF in the pathophysiology of the various manifestations of hypercortisolemic psychiatric syndromes associated with depression. Moreover, the overall work on CRF which has been reported in this annual report was presented in a paper submitted for the C.P. Richter Prize for Psychoneuroendocrinology which was awarded to Philip W. Gold in July of 1984.

2. Other Clinical Studies of Hypothalamic-Pituitary-Adrenal Function in Man

In addition to our studies of HPA function using CRF as a probe, we have devised other tests for examining the functional activity of this axis. One such paradigm is the pulsing of intravenous procaine, in collaboration with Drs. C. Kellner and R. Post. We note that intravenous procaine infusion causes an immediate and pronounced increase in ACTH secretion as well as synchronous secretion of beta-endorphin. To determine the locus of procaine action, we have explored the effects of intravenous procaine on the secretion of CRF into the CSF simultaneously with measurement of ACTH in a sheep model. Our data shows that, as noted above, procaine induces a subtle but clear rise in CSF CRF in association with significant increases in plasma ACTH, strongly suggesting that procaine-induced ACTH secretion into plasma occurs via activation of the hypothalamic CRF neuron. Of significance is the fact that our capacity to

stimulate hypothalamic CRF secretion with a benign test such as procaine infusion represents a major advance over the only other available test for hypothalamic CRF reserve, that is, the insulin tolerance test. Thus, the procaine stimulation test could potentially replace the insulin tolerance test as a standard test in clinical medicine for assessing the functional integrity of the hypothalamic neuron secreting CRF. Speculatively, since CRF has been shown to induce limbic seizures, procaine's effects on limbic electrical activity could be partially mediated by this peptide.

We have also evaluated HPA function by assessing the effects of opiate administration directly on ACTH and cortisol secretion, in addition to studying the effects of opiate pretreatment on the ACTH response to CRF. We have previously noted that acute I.V. methadone administration significantly depresses plasma cortisol secretion in depressed patients. In a more recent study, in collaboration with Dr. G. Chrousos, we have administered parenteral morphine to 11 volunteers and 16 patients with Cushing's disease and four patients with depression. We note that in normals, morphine significantly suppresses the circadian cortisol rise, the integrated cortisol level, and the frequency of secretory episodes. A phase delay of two hours was also noted in the cortisol surge in morphine patients. Patients with depression showed qualitatively similar responses, but those with Cushing's did not respond to morphine. In our studies examining morphine's effects on ACTH response to CRF with volunteers, we note that morphine causes a significant but modest blunting of the ACTH response to CRF. This suggests that morphine's inhibition of the hypothalamic-pituitary-adrenal axis occurs principally at a hypothalamic locus, since similar doses of morphine almost completely suppress basal plasma ACTH levels. Of theoretical interest is the fact that the effects of morphine on plasma cortisol secretion in normals is the inverse of the pattern of corticosteroid secretion seen in normals after continuous CRF administration. This suggests the possibility that a state of relative endorphin deficiency in depressed patients could result in hypersecretion of CRF and the manifest pattern of hypercortisolism seen in depressed patients.

We have also extensively studied urinary-free cortisol secretion in over 240 subjects and the findings have been reported in previous annual reports.

In collaboration with Dr. C. Kellner, we have attempted to elucidate a possible relationship between the increased ventricular brain ratio in depressed patients and hypercortisolism classically associated with depression. This study was undertaken in light of studies showing reversible enlargement of the ventricular brain ratio in various populations with hypercortisolism. Dr. Kellner noted a significant positive correlation between the magnitude of urinary-free cortisol excretion and the ventricular brain ratio.

3. Arginine Vasopressin (AVP)

We are continuing comprehensive investigation of arginine vasopressin function concentrating on studies of CSF AVP, the plasma AVP response to osmotic and nonosmotic stimulation, cognitive and behavioral response to AVP analog administration, and more recently, on the physiology of AVP secretion into the CSF. As noted in previous reports, we have extensively studied AVP function in a variety of neuropsychiatric illnesses, including anorexia

nervosa, primary affective disorder, and schizophrenia. Our previous work has shown a previously unidentified pattern of osmotic dysregulation in association with abnormalities of CSF AVP secretion in anorexia nervosa, subtle but significant reductions in plasma and CSF AVP secretion, and affective disturbance in association with significant effects of lithium and carbamazepine on these parameters, and complex abnormalities of osmoregulation in hyponatremic schizophrenic subjects in association with a subtle but significant decrease in CSF AVP secretion in this population. More recently, we have conducted dose-response studies of the effects of glucocorticoid antagonists' administration on AVP secretion, in light of the fact that there is a subtle reduction in the sensitivity and advance of the osmotic threshold of AVP secretion in depression. We note that antiglucocorticoid treatment causes a significant increase in plasma AVP secretion compatible with the idea that glucocorticoids exert tonic inhibition of central AVP function.

One focus this year has been on attempting to elucidate possible interrelationships between AVP secretion and CRF actions since AVP has been shown to markedly synergize CRF-induced ACTH secretion. An indirect study to assess this parameter was a study of the effects of CRF administration on ACTH secretion in patients with idiopathic diabetes insipidus, who lack circulating plasma AVP derived from nerve terminals in the posterior pituitary. These subjects actually showed exaggerated ACTH response to AVP, a finding which on the surface may seem surprising, but which is understandable in light of the fact that patients with DI, although deficient in the plasma, show elevated CSF levels of AVP and, presumably, in hypophyseal portal blood. This would be the case since neurons which secrete AVP into the CSF also send terminals to the floor of the median eminence. Thus, the exaggerated ACTH response to CRF in patients with diabetes insipidus supports the idea that AVP plays a physiologic role synergizing the effects of CSF. We have begun studies to determine whether AVP synergizes CRF effects on central nervous system function. For instance, a proposed study is to examine a possible role for central AVP administration on potentiating CRF-induced limbic seizures.

4. Oxytocin (OT)

As noted in previous reports, we have measured the level of oxytocin in CSF in a variety of psychiatric subgroups. One purpose for undertaking this study is that oxytocin is a structural analog of AVP and has been shown both centrally and at the pituitary corticotroph to antagonize AVP actions. We have established that OT is routinely present in the CSF of normal human subjects. A finding of no significant differences in levels of CSF OT between men and women is compatible with studies in experimental animals that estrogen does not influence the secretion of oxytocin into the CSF, in contrast to its effects on the level of OT in plasma. In psychiatric patients, we have extended our data which continues to support the previous finding that CSF OT is significantly reduced in drug-free manic patients, a finding which is the converse of the pattern seen with respect to AVP secretion. This finding of increased AVP and reduced OT in the CSF of manics is of interest in light of the reciprocal effects which AVP and OT have been reported to exert on cognition, REM sleep, hippocampal theta rhythms, and ACTH secretion. We also note that the level of OT is the reciprocal of that of AVP in anorexia nervosa; that is, whereas AVP levels are high in the CSF of underweight anorexics, OT levels are reduced.

We have also become increasingly interested in OT as a potential modulator of the pituitary-adrenal axis. It has been recently shown that OT administration significantly lowers basal plasma ACTH and interferes with AVP and insulin-induced hypoglycemia in ACTH secretion. We are currently exploring the hypothesis that OT may be a counterregulatory stress hormone and are examining its plasma secretion under a variety of circumstances, including studies in volunteers with a 48-hour sampling period.

5. Somatostatin

We are continuing to collaborate with Dr. D. Rubinow in studies of somatostatin secretion into the CSF. Dr. Rubinow has previously reported that somatostatin is significantly lower in a large group of drug-free unipolar and bipolar depressed patients compared to controls and that psychoactive agents such as carbamazepine and zimelidine significantly influence the levels of somatostatin in CSF. Dr. Rubinow has also shown significant relationships between CSF somatostatin secretion and the dexamethasone suppression status of patients, as well as relationships between urinary-free cortisol excretion and CSF somatostatin and depressed individuals.

6. Growth Hormone Releasing Factor (GHRF)

In collaboration with Drs. G. Merriam, G. Chrousos, and L. Loriaux, we have begun evaluating the pituitary somatotroph with bolus infusion of GHRF, a recently sequenced peptide with potent GH releasing properties. The studies with this peptide are currently in progress in patients with affective illness and anorexia nervosa.

Significance to Biomedical Research and to the Program of the Institute

The clinical work of the Unit on Clinical Neuroendocrinology focused on studies in volunteers, patients with major affective illness, anorexia nervosa, schizophrenia, and primary anxiety disorder, as well as in patients with Cushing's disease, ectopic ACTH secretion, and adrenal insufficiency. Moreover, extensive studies are conducted in laboratory animals and in primates. This work has resulted in elucidation of basic physiological mechanisms involving the secretion and actions of a variety of neuropeptides, including corticotropin releasing factor, vasopressin, oxytocin, and somatostatin. The focus on CRF has led to establishment of the pharmacokinetic properties and dose-response relationships with both ovine and human CRF in primates and human subjects, elucidation of the effects of continuous CRF infusion in volunteers, and the demonstration that ovine CRF is likely to be useful in diagnostic studies while human CRF is likely to be an important aid in studying pulsatile ACTH secretion. A safe and methodologically sound CRF stimulation paradigm has been developed, and data from this work has proved helpful in further elucidating the pathophysiology of hypercortisolism in depression and Cushing's disease and has resulted in a test which seems helpful in determining the differential diagnosis of these two entities. Moreover, CRF stimulation testing has also been shown to be clinically useful as an aid in differential diagnosis between Cushing's disease and ectopic ACTH secretion, and between various causes of adrenal insufficiency. To our knowledge, we have conducted the first series of studies elucidating the regulation of CRF secretion into the CSF, and have shown that anxiety producing agents such as

BCCE and R04864 cause a concomitant rise of CSF CRF and plasma ACTH, suggesting that CRF may play a role in the agitated behavior in these animal models. In other studies of hypothalamic-pituitary-adrenal regulation in experimental animals and in man, we have shown that i.v. procaine infusion, which is capable of causing limbic seizures and temporal lobe activation, causes a significant rise in plasma ACTH which in animal models is associated with a rise in CSF CRF. Thus, procaine may prove to be a more benign means of stimulating the CRF neuron in human subjects when compared to insulin-induced hypoglycemia. We have also continued studies on AVP secretion into the plasma and CSF compartments of patients with anorexia nervosa, affective illness and schizophrenia. In anorexia nervosa, we have described a new syndrome of abnormal osmoregulation invariably associated with abnormal AVP secretion into the CSF compartment. In affective illness, we have shown subtle defects in plasma and CSF AVP secretion. Moreover, we have also further elucidated the pharmacologic control of osmoreceptor function in man. Finally, we have introduced a new test of nonosmotic stimulation of AVP secretion, showing that a glucocorticoid antagonist causes a prompt significant rise in AVP secretion in human subjects. This study also confirms previous suspicions that glucocorticoids exert tonic inhibitory influences on AVP secretion in human subjects.

Proposed Course of Project

(1) We shall continue to intensely study the physiology, biological actions, and potential clinical significance of corticotropin releasing factor in psychiatric illness and in patients with frank endocrine abnormalities. We shall continue our clinical studies by establishing larger series, comparing the clinical utility of testing with ovine and human CRF. We shall also continue our pre-clinical studies to intensively study the regulation of CRF secretion into the CSF. The development of a specific and very sensitive human CRF assay will also allow us to study the secretion of this peptide into the CSF of our patient populations.

(2) We shall continue studies on the physiology of stress, applying a variety of probes, including CRF itself, benzodiazepine agonist and antagonist; and additional studies with new antigluccorticoid compounds which inhibit cortisol negative feedback and thus provide a further tool for examining the HPA axis. We shall also continue to explore the peripheral effects of CSF as a possible modulator of blood pressure during stressful situations.

(3) We shall begin testing of long-acting CRF agonists and antagonists which have been recently synthesized and made available to us by collaborators at the Salk Institute.

(4) We have commenced studies with other CRF factors which circulate in blood, including thymic hormones and so-called tissue CRF factors. A corollary project is the study of the relationship between hypothalamic-pituitary-adrenal function and immunologic function in our depressed and Cushing's populations, with a particular emphasis on exploring the biological effects of thymic hormones on HPA regulation.

(5) We have committed ourselves to extensively exploring the interrelationships between the opioids, arginine vasopressin, oxytocin, and CRF. A study which is well underway in this regard is examination of the simultaneous circadian rhythms of ACTH, beta-endorphin AVP and oxytocin in the plasma of human subjects and of these hormones and of CRF in the CSF of animal models.

(6) We plan to continue expansion of our laboratory facilities which provide essential support for our clinical and pre-clinical studies. This will allow not only further dissection of neuropeptide interrelationships in our clinical and pre-clinical studies, but also the capacity to integrate studies of the level of these peptides in biological fluids and tissue in association with elucidation of receptor function.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00180 - 02 BP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Psychobiology and Treatment of Menstrually-Related Mood Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

David R. Rubinow, M.D., Chief, Unit on Peptide Studies, BPB, NIMH
 Dr. Peter Roy-Byrne, Biological Psychiatry Branch, NIMH;
 Dr. Gerald L. Brown, Biological Psychiatry Branch, NIMH; Dr. Philip Gold,
 Biological Psychiatry Branch, NIMH; Dr. Norman Rosenthal, Clinical Psycho-
 biology Branch, NIMH; Dr. David Goldstein, Hypertension-Endocrine Branch,
 NHLBI; Dr. George Merriam, Endocrinology and Reproduction Research Branch,
 NICHD; Dr. Ronald Elin, Clinical Pathology Department, Clinical Center

COOPERATING UNITS (if any)

Biological Psychiatry Br., NIMH; Clinical Psychobiology Br., NIMH; Endocrinology &
 Reproduction Res. Br., NICHD; Hypertension-Endocrine Br., NHLBI; Clinical
 Pathology Department, CC

LAB/BRANCH

Biological Psychiatry Branch

SECTION

INSTITUTE AND LOCATION

NIMH - N.I.H. Bethesda, Maryland 20205

TOTAL MAN-YEARS:

2

PROFESSIONAL:

1

OTHER:

1

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The occurrence of dramatic changes in mood, behavior, cognition and somatic functioning in some women in relation to the menstrual cycle has recently been the focus of a great deal of public scrutiny. Yet despite fifty years of study, relatively little is known about the relationship between menstruation and disorders of mood. This project addresses itself to the major methodological difficulties characterizing earlier studies and is designed to study the psychobiology and treatment response of women with well defined menstrually-related mood disorders. The longitudinal screening methods employed in the first phase of our study appear capable of distinguishing women with menstrually-related mood syndromes from those who only believe that they have such a syndrome. We are currently measuring potential biological correlates of menstrually-related mood changes by assaying serial blood samples for relevant hormones and by performing neuroendocrine and electrophysiological tests during the symptom-free and symptomatic phases of the menstrual cycle. We are additionally performing double-blind controlled studies of several putative therapeutic agents including progesterone and pyridoxine.

The goals of this project are to detect and accurately describe menstrually-related mood disorders, explore their pathophysiology and response to pharmacological and environmental manipulation, and to document the relationship between reproductive endocrine change and disorders of mood as a way of further investigating the neurobiology of psychiatric illness.

I. Project Description

A. Objectives

This project has as its main intent the selection of subjects with carefully documented menstrually-related mood changes who can then undergo psychological and biological evaluation as well as participate in double-blind, placebo-controlled trials of several widely prescribed treatment modalities.

B. Methods Employed

1. Subjects

a. Subjects are self- and physician-referred women between the ages of 18 and 55 who complete visual analogue scale mood ratings twice daily for two months and on the basis of these ratings meet the following operational definition: a greater than 30% increase in self-rated anxiety or depression during the week prior to menses compared to the week following the cessation of menses in at least two consecutive cycles. All study participants are outpatients admitted to the outpatient division of the Section on Neuroendocrinology, Biological Psychiatry Branch.

b. Normal controls for this study include women with no complaints nor evidence of menstrually-related mood disorder and who are without primary psychiatric illness, and women who have complaints of, but no visual analogue scale evidence of, menstrually-related mood changes.

2. Procedures

Phase 1. The initial phase of this study is a screening phase involving subjects who are self- or physician-referred with complaints of severe changes in mood in apparent relation to the menstrual cycle. All subjects complete an extensive screening form which assesses the frequency and severity of symptoms in relation to menstruation as well as past and present psychiatric history, social history, family history, medical and GYN history, and medication history. Subjects are also provided with three months' worth of visual analogue scales for anxiety and depression which they are asked to complete on a twice-daily basis. Finally, subjects are individually interviewed in order to more adequately assess menstrually-related phenomenology, medical history, and psychiatric history, with all patients administered the schedule for affective disorders and schizophrenia interview in order to produce a lifetime psychiatric diagnosis.

Phase 2. This is an intensive psychobiological evaluation phase for patients meeting entry criteria for the study.

a. Patients are given a thorough physical and laboratory examination in order to rule out the presence of unknown medical illness.

b. Plasma steroid and peptide studies. Fasting 8:00 a.m. blood samples are obtained at nine points during a menstrual cycle in order to evaluate the levels, relative concentrations, and pattern of secretion of several peptide and steroid hormones which have been implicated in menstrually-related mood dis-

orders such as estrogen, progesterone, aldosterone, and beta-endorphin. These studies are being performed in collaboration with Drs. George Merriam (ERR, NICHD) and Philip Gold (BPB, NIMH).

c. Plasma catecholamines. Because of the putative role of central catecholaminergic function in affective disorder and because of evidence that plasma norepinephrine concentrations vary in synchrony with the menstrual cycle, we are measuring plasma norepinephrine, epinephrine, and DOPAC in collaboration with Dr. David Goldstein (HE, NHLBI).

d. Plasma magnesium. Because of reports of altered monocyte magnesium levels in women with "premenstrual tension," we are measuring plasma monocyte magnesium concentrations at two points during both the follicular and luteal phases of the menstrual cycle in collaboration with Dr. Ronald Elin (CP, CC).

e. Additional neuroendocrine measures. In order to assess whether specific neuroendocrine abnormalities accompany the symptomatic phase in women with menstrually-related mood disorders, we are performing two well described (dexamethasone suppression test and TRH stimulation test) and one recently described (CRH stimulation test) neuroendocrine tests employed in affective disorder studies. The dexamethasone suppression test and TRH stimulation test are both performed in routine fashion during both the symptom-free and symptomatic phases of the menstrual cycle. The corticotropin releasing hormone (CRH) stimulation test is performed in collaboration with Dr. Philip Gold and involves administering five micrograms per kilogram of CRH in bolus form followed by two hours of periodic blood sampling.

f. Psychometrics. In addition to conventional self-ratings of anxiety, depression, and mood during blood sampling days, each patient completes on a twice-daily basis computer scanable visual analogue scales for depression, anxiety, fatigue and global assessments and keeps a sleep log on a daily basis. A twenty-one item assessment form developed by Dr. Jean Endicott specifically for patients with menstrually-related disorders is being used by our patient group. In collaboration with Dr. Gerald L. Brown (BPB, NIMH), MMPI's are completed by all patients in the second phase of the study. Records of frequency and perception of stressful events are being completed under the supervision of M. Christine Hoban, M.S.W., and a method for assessing life events and external stressors has been developed by Dr. Peter Roy-Byrne (BPB, NIMH). Cognitive batteries, including the Stroop Color Discrimination Test and the Digit-Symbol Substitution Test, are being administered during symptomatic and symptom-free phases by M. Christine Hoban (BPB, NIMH).

Phase 3. This is a multi-modality treatment phase for patients who have completed Phase 2.

a. Pharmacologic. Double-blind, placebo-controlled crossover evaluations of progesterone, medroxyprogesterone acetate, pyridoxine, and carbamazepine are currently being conducted. The first three agents mentioned are cited as effective in the literature but have not been systematically demonstrated to be more effective than placebo in studies to date, largely as a function of methodological flaws which render the results of these studies ungeneralizable. Carbamazepine has been successfully used in the treatment of pre-menstrual psychomotor seizure-related behavioral syndromes as well as major affective disorder.

C. Findings

Forty-three percent (69/160) of subjects who have completed three months of daily ratings met operational criteria for a menstrually-related mood disorder. Comparison of the prevalence of life-time psychiatric history in those women with and without confirmed menstrually-related mood disorders revealed a significant difference between these groups, with the difference largely attributable to the excess psychiatric morbidity in those women in whom we are unable to confirm the existence of a menstrually-related mood disorder. These findings largely invalidate many results from earlier studies which did not prospectively confirm the diagnosis of premenstrual syndrome in the population prior to study and therefore utilized a heterogeneous sample that was unrepresentative of the population with premenstrual syndrome. Assessment of retrospective items from an extensive screening questionnaire revealed no differences between those in whom the syndrome could be confirmed and those without prospective confirmatory evidence of the syndrome. We have established that the computer-scanable rating forms developed in collaboration with Dr. Norman Rosenthal are effective in assessing symptomatic fluctuation in relation to menstrual cycle. Thirty-five women and five controls have completed the second phase of the protocol, participating in one month of frequent blood drawings for assessment of endocrine variation over the course of the menstrual cycle as well as in assessment of cognitive function and response to dexamethasone and TRH infusion during the symptomatic and symptom-free phases of the cycle. Preliminary evaluation of endocrine factors in women with premenstrual syndrome reveals no inadequate corpus luteal activity and no systematic hormonal deficiency. Confirmation of these findings must await hormonal assessment of the remaining subjects and controls. Assessment of TSH response to TRH in patients and controls during the symptomatic and symptom-free phases revealed no differences in TSH stimulation. However, exaggerated prolactin responses to TRH infusion have been observed in a number of patients. The significance and consistency of these findings remain to be established. Assessment of white cell, red cell and plasma magnesium levels over the course of the menstrual cycle revealed marked nonsystematic variation in RBC and WBC magnesium levels in the luteal compared with the follicular phases. Assessment of plasma catecholamines showed no systematic or symptom-related variation. Perceptions of cognitive performance varied considerably with menstrual cycle phase, although actual performance revealed no systematic menstrual-cycle phase-related alterations.

While ten people have entered the treatment phase of the study, no one has completed the ten month trial at this point.

D. Proposed Course of Project

To date over 500 women have requested to be participants in our project and are at various stages of evaluation. With a group of well defined patients, we hope to explore the natural course of menstrually-related mood disorders as well

as their phenomenology and biological correlates in relation to treatment response. Early endocrine findings will be pursued and treatment protocols completed. Cognitive testing will be continued with the addition of distractors during testing. In addition we wish to expand our investigation of the effects of menstrual phase on mood to include patients hospitalized at the Clinical Center with major affective disorder, panic anxiety disorder, anorexia-bulimia as well as patients with hereditary angioedema. Our early experience with a number of women with these disorders suggests that symptoms may be exacerbated or may cluster during the premenstrual period; these clinical impressions require prospective confirmation.

E. Significance to Biomedical Research and the NIMH Intramural Research Program

Despite the current lack of clear understanding of the nature of the relationship between mood disorders and the menstrual cycle, numerous studies of this phenomenon suggest its importance to the psychiatrist on many levels: practically (as a problem about which the psychiatrist may be called to consult or as a factor which may influence the course of the treatment of patients); heuristically (as a model for learning about state changes, a process of clear relevance to studies of other mood state disorders such as manic-depressive illness or panic anxiety disorder); and conceptually (as a potential means for providing biological-phenomenological isomorphs and further understanding the role of entrainment in episodic or cyclic psychiatric disorders). Menstrually-related mood disorders in their own right are important to better understand, if only for the fact that there are large numbers of women who feel that they suffer from such syndromes and seek treatments which are unproved and potentially dangerous. In addition it would appear that menstrual cycle phase is a variable which has been all too frequently ignored in studies of traditional psychiatric and medical illnesses. It is our belief, therefore, that this project will provide information that will be of immediate clinical relevance and that will further our understanding of the complex relationship between endocrine system activity and mood.

Publications:

Rubinow, D.R. and Roy-Byrne, P.P.: Premenstrual syndromes: overview from a methodologic perspective. Am. J. Psychiatry 141: 163-172, 1984.

Rubinow, D.R., Roy-Byrne, P.P., Hoban, M.C., Gold, P.W., and Post, R.M.: Prospective assessment of menstrually-related mood disorders. Am. J. Psychiatry 141: 684-686, 1984.

Rubinow, D.R., Roy-Byrne, P.P., and Hoban, M.C.: Menstrually-related mood disorders. In Phillips, A. and McGuire, J.L. (Eds.): Premenstrual Tension and Dysmenorrhea. Baltimore, Williams & Wilkins, in press.

Rubinow, D.R., Roy-Byrne, P.P., Hoban, M.C., Grover, G., and Post, R.M.: Premenstrual syndromes: past and future research strategies. Am. J. Psychiatry, in press.

Rubinow, D.R., Roy-Byrne, P.P., Hoban, M.C., Grover, G., and Post, R.M.: Menstrually related mood disorders. American Psychiatric Press, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00181 - 01 BP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Hormonal Studies of Affective Disorder

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

David R. Rubinow, M.D., Chief, Unit on Peptide Studies, BPB, NIMH
 Dr. David Pickar, NSB, NIMH; Dr. Allen Doran, NSB, NIMH; Dr. George Merriam, ERRB,
 NICHD; Dr. Thomas Insel, CNB, NIMH

COOPERATING UNITS (if any)

Endocrinology of Reproduction Res. Br., NICHD; Clinical Neuroscience Br., NIMH;
 Clinical Neuropharmacology Br., NIMH.

LAB/BRANCH

Biological Psychiatry Branch

SECTION

INSTITUTE AND LOCATION

NIMH - N.I.H. Bethesda, Maryland 20205

TOTAL MAN-YEARS:

2

PROFESSIONAL:

1

OTHER:

1

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Endocrine studies were performed to assess alterations in somatostatin and cortisol activity in relation to affective illness. The relationship between depression-related cognitive disorder and endocrine dysregulation has also been examined. a. Somatostatin - With recently learned assay techniques and in collaboration with Drs. Pickar and Doran, we confirmed a predicted relationship between post-dexamethasone cortisol and CSF somatostatin. This relationship may provide a biochemical explanation for psychiatric disorder related cortisol abnormalities. Work is currently underway to clarify the significance of these findings as well as to further delineate the factors regulating hypothalamic somatostatin secretion. b. Cortisol - We have investigated a number of measures of hypothalamic-pituitary-axis activity including response to dexamethasone, mean-urinary free cortisol excretion, and the relationship between plasma cortisol levels and affective state in relation to a variety of biological and phenomenological features of depression. We have demonstrated clear cut elevations in CSF cortisol in depressed patients relative to normal controls and patients during the improved state. We have reported a relationship between cortisol hypersecretion and a measure of the cognitive impairment seen in depression (Halstead Category Test errors). We have additionally reported effects of the anti-convulsant psychotropic agent, carbamazepine, on the cortisol system including carbamazepine-induced escape from dexamethasone suppression and induction of cortisol hypersecretion in patients who under basal conditions excrete normal amounts of mean urinary free cortisol. c. Cognition - In addition to the findings mentioned above, we have demonstrated specific deficits in verbal and non-verbal affect recognition in patients with depression using an instrument (Face Test) validated in patients with neurological lesions. The goals of the above listed projects will be to expand our understanding of known relationship between depression and endocrine function with specific emphasis on cognitive functioning.

I. Project Description

A. Objectives

The goal of this project is to study measures of two endocrine systems, somatostatin and cortisol, in patients with affective illness in order to expand our understanding of the mechanisms and significance of reported abnormalities in these systems in affective illness.

B. Methods Employed

1. Subjects

a. Subjects are inpatients on a NIMH affective disorder unit meeting criteria for major depressive disorder.

b. Normal controls for CSF and hormone infusion studies are subjects participating in the normal volunteer program at the NIH.

2. Procedures

Lumbar punctures are performed to obtain CSF samples for somatostatin, cortisol, and other related CNS peptides/neurotransmitters. Additionally, blood and urine samples are obtained for measurement of cortisol. Dexamethasone suppression tests are performed in patients while medication free and while on treatment with carbamazepine. Infusions of oxytocin and vasopressin have been performed in order to assess the effects of these hormones on cortisol secretion and cognitive functioning.

C. Findings

Evidence of affective state-related cortisol dysregulation has been found with increased mean urinary free cortisol excretion during depression, increased CSF cortisol during depression, and significant relationships between plasma cortisol and affective state observed. Vasopressin dramatically stimulated cortisol secretion in both normal volunteers and patients with major affective illness, with no diagnosis-related differences observed. In collaboration with Drs. Pickar and Doran, we observed significantly lower CSF somatostatin in both schizophrenic and depressed patients showing premature escape from dexamethasone suppression.

A central stimulating as well as a peripheral metabolic effect of carbamazepine has been proposed as the mechanism for its induction of escape from dexamethasone suppression, on the basis of observations of increased mean urinary free cortisol on carbamazepine in patients who excrete normal amounts of cortisol at baseline. Our further demonstration of the reduction of CSF somatostatin by carbamazepine suggests reduced somatostatin as an additional possible mechanism for carbamazepine-induced escape from dexamethasone suppression.

In addition to our report of the relationship between cognitive impairment (Halstead Category Test errors) and mean urinary free cortisol excretion in depressed patients, we have demonstrated the utility of a measure of verbal and

nonverbal affect recognition (Face Test) in identifying cognitive impairment in patients with affective disorder. Cognitive processing in the form of recognition recall appeared to be enhanced after 24 hours in a small number of patients by vasopressin administration. No disruptive effect of oxytocin was noted in the same paradigm.

D. Proposed Course of Project

We hope to:

1. Expand the known clinical concomitants of various measures of hypothalamic-pituitary-adrenal axis dysregulation;
2. Establish, in collaboration with Dr. Thomas Insel, the impact of reduced peripheral levels of somatostatin on pituitary ACTH release;
3. Explore, with Dr. George Merriam, those factors regulating hypothalamic somatostatin activity;
4. Pursue in a larger population of depressed patients the memory enhancing effects of vasopressin infusion;
5. Investigate the neuroendocrine concomitants of impaired performance on the Halstead Category Test and the Face Test.

E. Significance to Biomedical Research and the NIMH Intramural Research Program

Depression-related dysregulation of somatostatin and cortisol may provide a window into the central neurochemical lesions responsible for depression. Further, specific behavioral or physiological disturbances (e.g., cognitive impairment or cortisol dysregulation) may be products of abnormal neuroendocrine activity. It may prove to be the case that depression-related reductions in somatostatin are mechanistically relevant to depression-related disturbances in hypothalamic-pituitary-adrenal activity, the most commonly reported biological abnormality in depression. Further study may not only enhance our knowledge of the neurobiology of depression but may, as well, more generally inform us about the relationship between hormones and human behavior.

Publications:

Rubinow, D.R., Gold, P.W., Post, R.M., Ballenger, J.C., and Reichlin, S.: CSF somatostatin in affective illness. Psychopharmacol. Bull., 19: 422-425, 1983.

Rubinow, D.R., Gold, P.W., Ballenger, J.C., and Post, R.M.: Somatostatin in patients with affective illness and in normal volunteers. In Post, R.M. and Ballenger, J.C. (Eds.): Neurobiology of Mood Disorders. Baltimore, Williams & Wilkins, 1984, pp. 369-387.

Rubinow, D.R., Post, R.M., Gold, P.W., Ballenger, J.C., and Wolff, E.A.: The relationship between cortisol and the clinical phenomenology of affective illness. In Post, R.M. and Ballenger, J.C. (Eds.): Neurobiology of Mood Disorders. Baltimore, Williams & Wilkins, 1984, pp. 271-289.

Rubinow, D.R., Post, R.M., Savard, P., and Gold, P.W.: Cortisol hypersecretion and cognitive impairment in depression. Arch. Gen. Psychiatry 41: 279-283, 1984.

MacDonald, E., Rubinow, D.R., and Linnoila, M.: Variations in the sensitivity of red blood cell membrane Ca^{2+} -ATPase to calmodulin stimulation in patients with bipolar affective disorder. Arch. Gen. Psychiatry 41: 487-493, 1984.

Rubinow, D.R., Post, R.M., Gold, P.W., Uhde, T.W., Ballenger, J.C., and Reichlin, S.: Effects of carbamazepine on cerebrospinal fluid somatostatin in patients with major affective illness. In Porter, R.J. (Ed.): Advances in Epileptology, Vol. XV. New York, Raven Press, 1984, pp. 49-52.

Rubinow, D.R., Post, R.M., and Gold, P.W.: Neuroendocrine and peptide effects of carbamazepine: clinical and mechanistic implications. Psychopharmacol. Bull., 20: 590-594, 1984.

Rubinow, D.R., Gold, P.W., Ballenger, J.C., Cowdry, R., and Post, R.M.: CSF somatostatin in affective illness. Prog. Neuropsychopharmacol. Biol. Psychiatry, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00182 - 01 BP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Behavioral Medicine

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

David R. Rubinow, M.D., Chief, Unit on Peptide Studies, BPB, NIMH
 Dr. Russell Joffe, Biological Psychiatry Branch, NIMH; Dr. David Pickar, Biological Psychiatry Branch, NIMH; Dr. William Sindelar, Surgery Branch, NCI; Dr. Philip Schneider, Surgery Branch, NCI; Dr. Allan Mirsky, Laboratory of Psychology and Psychopathology, NIMH; Dr. Clifford Lane, Lab. of Immunoregulation, NIAID; Dr. Anthony Fauci, Laboratory of Clinical Investigation; NIAID; Dr. Dan Longo, Medicine Branch, NCI; Dr. Henry Masur, Critical Care Medicine, Clinical Center; Dr. James Hathorn, Pediatric Oncology Branch, NCI; Dr. Gary Peck, Derm. Br., NCI;

COOPERATING UNITS (if any)

BPB, LPP, CPB, NSB, NIMH; SB, MB, PB, D, NCI; LIR, LCI, NIAID; CCM, PHARM, CC; CE, DD, OPPA, NIADK; MD, NHLBI

LAB/BRANCH

Biological Psychiatry Branch

SECTION

INSTITUTE AND LOCATION

NIMH - N.I.H. Bethesda, Maryland 20205

TOTAL MAN-YEARS:

2

PROFESSIONAL:

1

OTHER:

1

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Advances in neuroscience as well as the appreciation of the often unrecognized role of behavior and disturbances of behavior in many medical disorders has led to the creation of a behavioral medicine research program based in the Consultation-Liaison Service of the Intramural Program. Ten protocols are currently active or planned investigating the mood, cognitive and behavioral concomitants of cancer of the pancreas, acquired immune deficiency syndrome, interferon therapy, metoclopramide administration, steroid therapy, and thyroid hormone replacement and withdrawal. These protocols will address such areas as: a) the effects of previous psychiatric history on the psychiatric morbidity associated with certain diseases and their treatment; b) the psychiatric phenomenology of certain diseases and their treatment; c) the treatment response characteristics of psychiatric disorders associated with diseases or their treatment; d) biochemical factors that may serve as predictive diagnostic markers for illness or for treatment-associated mood/behavioral or cognitive syndromes. The goals of this project will be to address these areas of investigation in selected patient populations at the N.I.H. Clinical Center.

COLLABORATORS:

Dr. Patricia Petrick, Clinical Endocrinology Branch, NIADDDK
Dr. Jacob Robbins, Clinical Endocrinology Branch, NIADDDK
Dr. Robert Golden, Clinical Psychobiology Branch, NIMH
Dr. Jeffrey Hoeg, Molecular Disease Branch, NHLBI
Dr. Bryan Brewer, Molecular Disease Branch, NHLBI
Dr. Daniel Hommer, Clinical Neuroscience Branch, NIMH
Dr. Steven Paul, Clinical Neuroscience Branch, NIMH
Dr. Owen Wolkowitz, Clinical Neuroscience Branch, NIMH
Dr. Jay Hoofnagel, Digestive Diseases Branch, NIADDDK
Dr. E. Anthony Jones, Digestive Diseases Branch, NIADDDK
Dr. Pierre Renault, Associate Director, NIADDDK

I. Project Description

A. Objectives

This project has as its main intent the identification of mood and cognitive factors that appear in the context of specific medical illnesses and their treatment, determination of the relationship between these factors and both the primary medical disorder and prior psychiatric history, and utilization of the occurrence of these factors in a medical context as models for the production of similar symptoms in a primarily psychiatric context.

Protocols

Active:

- 1) Clinical and Biological Features of Mood and Cognitive Disorders in Patients with Carcinoma of the Pancreas.
- 2) Neuropsychiatric Dysfunction in Patients with Acquired Immune Deficiency Syndrome (AIDS).
- 3) Psychiatric Effects of Treatment of Cystic Acne with 13 - Cis-Retinoic Acid.
- 4) Longitudinal Assessment of Cognitive and Mood Disorders in Patients with Type V Hyperlipoproteinemia.
- 5) Mood and Cognitive Effects of Interferon Administration in Patients with Chronic Active Hepatitis.

Written:

- 1) Assessment of Neuropsychiatric Concomitants of Metoclopramide Administration.
- 2) A Prospective Study of the Behavioral, Cognitive and Neurochemical Effects of Chronic Systemically Administered Corticosteroids.
- 3) The Effect of Thyroid Replacement and Withdrawal on Cognition and Mood in Patients with Carcinoma of the Thyroid.

Planned:

- 1) Neuropsychiatric Evaluation of Peripheral Thyroid Hormone Resistance and Its Treatment with Triiodothyronine.
- 2) Evaluation of Effects of Proglumide on Development of Tolerance to Opiate Analgesia.

B. Methods Employed

1. Subjects

a. Subjects are NIH patients who are referred for participation in these protocols by collaborators from the Institute responsible for the primary care and treatment of these patients.

b. Controls for the individual studies are selected in a way that allows for stratification of populations with respect to the relevant variables under study. For example, assessment of the incidence of endogenomorphic depression in patients with carcinoma of the pancreas requires utilization of a patient control population with other intra-abdominal malignancies.

2. Procedures

a. Psychiatric Diagnostic Evaluation

The primary methodology employed is that of evaluating the psychiatric history of all subjects and their families utilizing a semistructured psychiatric interview, the Schedule for Affective Disorders and Schizophrenia (SADS-L) which provides information from which an RDC diagnosis can be made.

b. Longitudinal Evaluation

Most studies utilize a "self as own control" design employing longitudinal assessment of mood ratings, physical symptoms, and cognitive performance. Measures of mood include the Beck Depression Inventory, the State-Trait Anxiety Inventory, the Symptom Check List-90, and a number of hundred millimeter line visual analogue scales of mood. Visual analogue scales are also used to assess the presence and severity of physical symptoms. Cognitive measures include the Mini-Mental Status Exam, the Halstead-Wepman Test, and an extensive battery of neuropsychological tests developed by Kathleen Squillace (LPP, NIMH). In most instances, episodic observer ratings are augmented by daily subjective ratings. By these means, the time course of the development of cognitive or affective changes can be more precisely defined.

c. Laboratory Assessment

Urine and/or blood samples are collected in order to permit evaluation of those biological substances believed to be related to the development of affective or cognitive disturbances.

3. Findings

Preliminary findings include:

- 1) Marked diminution in anxiety in patients successfully treated for cystic acne;
- 2) Uniform escape from dexamethasone suppression seen in patients with carcinoma of the pancreas, with more sporadic escape seen in patients with other intra-abdominal malignancies;
- 3) The existence of pronounced cognitive deficits in patients with early stage Acquired Immune Deficiency Syndrome (AIDS).

C. Proposed Course of Project

The active and proposed studies noted above will be continued until adequate numbers of subjects are obtained. Early findings in these studies should permit design of focused investigations of the neurobiology of specific mood, behavioral and cognitive disorders.

D. Significance to Biomedical Research and the NIMH Intramural Research

Program

The studies in this project are hypothesis-generating as well as hypothesis-testing. Thus, they should not only help to expand the behavioral phenomenology of many medical disorders, but should as well suggest optimal studies for the application of modern neuroscientific techniques to disorders of regulation of mood and cognition. The utilization of medical disorders as models for the development of mood and cognitive disturbances in the context of biological dysregulation should clarify the meaning of those biological alterations already observed in psychiatric disorders.

Publications:

Rubinow, D.R.: Psychophysiologic symptoms - headache. In Leigh, H. (Ed.): Psychiatry in the Practice of Medicine. Menlo Park, California, Addison-Wesley, 1983, pp. 171-188.

Rubinow, D.R.: Research at the interface. Gen. Hosp. Psychiatry 5: 99-103, 1983.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00124-07 BP

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanisms of Action of Lithium in the Treatment of Affective Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Agu Pert, Ph.D., Chief, Unit on Behavioral Pharmacology, BPB, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Biological Psychiatry Branch

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Unit on Behavioral Pharmacology

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NIMH, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Work on this project has been temporarily postponed pending the establishment of an appropriate autoradiographic facility and the acquisition of autoradiographic skills by laboratory personnel.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00147-09 BP

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Behavioral and

Physiological Effects of Brain Peptides and Other Psychoactive Compounds

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Agu Pert, Ph.D., Chief, Unit on Behavioral Pharmacology, BPB, NIMH

Thomas Seeger, Staff Fellow; Paul B.S. Clarke, Fogarty Fellow; Susan Weiss, Staff Fellow, BPB, NIMH; C.C. Chiueh, Staff Fellow, R.U. Esposito, Staff Fellow, LCS, NIMH; L.J. Porrino, Staff Fellow; L. Sokoloff, Chief, LCM, NIMH

COOPERATING UNITS (if any)

Laboratory of Cerebral Metabolism, NIMH
Laboratory of Clinical Science, NIMH

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Unit on Behavioral Pharmacology

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:

6.0

PROFESSIONAL:

5.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Autoradiographic visualization of nicotine receptors labeled with either ^3H -nicotine or ^3H -acetylcholine in the presence of atropine revealed high concentrations in the specific thalamic nuclei, interpeduncular nucleus, superior colliculus, substantia nigra, molecular layer of the dentate gyrus, presubiculum and laminae III/IV of the cerebral cortex. Labeling of nicotine receptors with alpha-bungarotoxin revealed an entirely different pattern. Binding was highest in the cerebral cortex, hypothalamus, hippocampus, inferior colliculus and in certain brainstem nuclei, suggesting the presence in brain of two distinct subtypes of the nicotine receptor. Lesions of the habenula decreased the binding of ^3H -nicotine in the interpeduncular nucleus. Lesions of the medial forebrain bundle decreased the binding of ^3H -nicotine in the zona compacta of the substantia nigra suggesting the presence of nicotine receptors on dopamine cell bodies. Systemic injections of nicotine increased the firing rate of dopaminergic zona compacta neurons in the substantia nigra suggesting that nicotine can influence dopaminergic neural activity. Injections of phencyclidine (PCP) into the nucleus accumbens produced locomotor excitation which was blocked by systemic administration of haloperidol. Injections of PCP into periaqueductal gray matter and mesencephalic reticular formation produced decreases in locomotor output. Calcitonin produced patent analgesic effects following injections into the periaqueductal gray matter and reticular formation. Electrical stimulation of the arcuate nucleus produced a naloxone reversible analgesic effect accompanied by decreases in binding of ^3H -diprenorphine in the terminal regions of the endorphin system suggesting the release of endorphins by this manipulation. Employing local cerebral glucose utilization (LCGU) procedures, rewarding brain stimulation of the ventral tegmental area and the substantia nigra divergent and convergent patterns of metabolic activation were observed.

Project Description

Objectives

Autoradiographic Distribution of Nicotine Receptors

Biochemical, electrophysiological and behavioral evidence suggest that nicotine acts centrally. These effects are probably mediated through specific nicotine receptors in brain. Stereospecific, saturable and reversible binding of tritiated nicotine to rodent brain membranes has been demonstrated. Binding is characterized by a high affinity for nicotine and was displaced preferentially by nicotine agonists including acetylcholine. The autoradiographic distribution of nicotine receptors has not yet been described. In this study the autoradiographic distribution of [^3H]-nicotine binding was assessed in slide mounted rat brain sections prepared with standard procedures. Biochemical experiments were also performed on the brain sections which were scraped off the slides.

The putative central nicotinic receptors have been labeled using various other ligands with known peripheral actions. Alpha-bungarotoxin (BTX) is probably the most widely used radio ligand. Binding of this compound is saturable and reversible, and is displaced preferentially by nicotinic agents including nicotine and acetylcholine. As noted above, high affinity binding of agonists to putative nicotinic cholinergic receptors in rodent brain membranes has also been demonstrated. Studies have employed either [^3H]-nicotine or [^3H]-acetylcholine in the presence of excess atropine to block muscarinic cholinergic receptors. Although nicotinic receptors labeled by these methodologies appear to possess differential distribution across microdissected brain areas, there is little consensus among studies. There appears to be a consistent lack of correlation between the regional distribution of [^3H]-nicotine and ^{125}I -BTX binding in mouse brain. In this study we compared the autoradiographic distribution of ^3H -nicotine, ^3H -ACh in the presence of atropine and ^{125}I -BTX.

Effects of Lesions on [^3H]-nicotine Binding

One possibility is that the high affinity nicotine receptors are localized on cholinergic neurons and regulate their activity, perhaps as part of a feedback mechanism. To test this possibility rats were lesioned in two regions of the brain from which originate cholinergic projections. One region of the brain that was lesioned was the habenula which sends cholinergic projections to the interpeduncular nucleus. In our previous studies this nucleus was shown to have considerable binding of ^3H -nicotine. The other area lesioned was the laterodorsal tegmental nucleus which sends a cholinergic input to the anteroventral thalamus. Sections were taken through both of these terminal fields following the lesion and processed for autoradiographic visualization of ^3H -nicotine binding using a tritium-sensitive film.

Nicotine and Nigrostriatal DA Function

There is a possibility that nicotine may modulate DA activity. Several studies were initiated to assess the interactive effects between nicotine and the

nigrostriatal DA pathways. Single cell recording techniques were used to evaluate whether nicotine alters the spontaneous activity of dopaminergic zona compacta neurons in the substantia nigra following systemic injections. One region of the brain that appeared to have an abundance of nicotine receptors was the substantia nigra. Attempts were made to ascertain whether the high affinity nicotine receptors described above were located on the dopamine cell bodies in the substantia nigra. Lesions were made in the medial forebrain bundle (the pathway through which nigrostriatal DA neurons project to the caudate nucleus) with 6-hydroxydopamine. If nicotine receptors are localized on either the cell bodies or terminals of the dopamine neurons, their ^3H -nicotine binding should decrease ipsilateral to the lesion in the zona compacta of the substantia nigra and caudate nucleus, respectively. The rotational model was also used to evaluate whether acute and chronic nicotine enhances activity of the dopaminergic nigrostriatal pathways. Rats were lesioned unilaterally in the substantia nigra with 6-OHDA. Two weeks later they were injected with either 0.1, 0.2, 0.4 or 0.8 mg/kg of nicotine tartrate and placed in automated rotometers. Following the determination of the dose-response function the animals were divided into two groups. One group was injected daily for two weeks with nicotine and the other group was injected with saline. At the end of this period, both groups were tested in the rotometers following an injection of nicotine.

Effects of Phencyclidine on Locomotor Activity Following Intracerebral Injections

Phencyclidine (PCP) has been reported to have significant effects on locomotor behavior. Low doses appear to stimulate locomotor output while high doses result in an initial depression which is followed by excitation. Locomotor excitation induced by PCP may involve the dopamine (DA) system since DA receptor blockers are effective in attenuating this response. Little more, however, is known regarding the precise mechanisms or loci of action of PCP in producing alterations in locomotor behavior. The purpose of these studies was to ascertain the loci of action of PCP in brain in relation to locomotor output and to define its mechanism of action. In the first study, rats were implanted with cannulae guides aimed for the lateral ventricle. PCP (5,25,75 nmoles) was injected intraventricularly and locomotor activity was measured over the next three hours. In the next series of studies, rats were implanted with cannulae guides aimed for the ventral thalamus, caudate nucleus, ventral tegmental area, nucleus accumbens, periaqueductal gray matter and mesencephalic reticular formation. The effects of PCP on locomotor activity were assessed following injections into these structures.

Analgesic Effects of Non-Opiate Peptides

Opiate peptides as well as opiate alkaloids are known to induce their analgesic effects through the periaqueductal gray matter (PAG). For example, injections of opiates into the PAG, which is high in opiate receptors and opiate peptides, produce profound analgesia in the rat. Besides endorphins, the PAG also contains relatively high concentrations of other neuropeptides and neuropeptide receptors. Previously we have demonstrated that a number of neuropeptides, including neurotensin, VIP and bombesin, induce analgesia following injections into the PAG. We have recently found that calcitonin,

another neuropeptide which has a remarkably high receptor distribution in the PAG, also produces long lasting analgesia in the rat. In this series of studies we have attempted to localize the analgesic effects of calcitonin by evaluating the analgesic reactions following microinjections into discrete areas of the rat brain.

Metabolic Mapping of Nigral Connections and Circuitry Underlying Rewarding Brain Stimulation with the 2-[¹⁴C] Deoxyglucose Method

Rats will self-stimulate with electrodes in either the ventral tegmental area (VTA) or substantia nigra zona compacta of the mesencephalon. These anatomically continuous regions of the brain have different patterns of afferent and efferent connections. In this series of studies we used the quantitative 2-[¹⁴C] deoxyglucose autoradiographic method to compare and contrast the patterns of local cerebral metabolic activity that result from self-stimulation to these two areas. In a second series of studies we evaluated the patterns of local cerebral metabolic activity that were either allowed to self-stimulate to the ventral tegmental area, were given experimenter-administered stimulation at the subject's preferred rates, or that received no stimulation. In a final review of studies we evaluated the effects of amphetamine on rats responding for intracranial self-stimulation to the VTA. The following four groups were compared: 1) high current: rats self-stimulating at an amplitude of 250-200 u amps; 2) low current and amphetamine: rats self-stimulating at 100 u amps given 0.5 mg/kg d-amphetamine; 3) amphetamine alone: rats given 0.5 mg/kg d-amphetamine but not allowed to self-stimulate; 4) control with rats which did not receive amphetamine or stimulation.

Unilateral electrical stimulation of the substantia nigra, zona compacta and zona reticulata increases local cerebral glucose utilization (LCGU) in the ipsilateral globus pallidus, entopeduncular and subthalamic nuclei. LCGU is reduced in the corresponding lateral habenula. Using a computer enhanced imaging system we have assessed stimulation in animals with and without 6-OHDA lesions of the ipsilateral medial forebrain bundle.

Visualization of Changes in Opiate Receptor Occupancy Due to Brain Stimulation

We have recently described a novel in vivo autoradiographic method which allows the indirect visualization of functional opiate peptide release, based on the assumption that prior receptor occupation will exclude the binding of an exogenously applied tritium-labeled, opiate ligand. Coupled with tritium-sensitive film autoradiography, it allows the mapping of relative levels of behavior-specific receptor occupancy throughout the brain. We have found two types of these manipulations which are known to release endorphins: forced swims in cold water and prolonged intermittent footshock. Both of these manipulations were found to enhance release of endorphins in a variety of areas of the brain. In this study we attempted to evaluate the effects of electrical stimulation of the cell body area of the beta-endorphin system (arcuate nucleus) and the periaqueductal gray matter on opiate release utilizing the new in vivo technique. In the same rats we also assessed the increase in nociceptive thresholds induced by stimulation of these two areas and whether the alterations in nociceptive thresholds were reversed by naloxone, the opiate antagonist.

Major Findings

No clear qualitative differences were found in the patterns of ^3H -ACh and ^3H -nicotine labeling in rat brain slices, whereas ^{125}I -BTX bound with a completely different distribution. The autoradiographs of ^3H -ACh and ^3H -nicotine demonstrated high densities of labeling in the following structures: interpeduncular nucleus, all thalamic nuclei except posterior and intralaminar, superior colliculus and medial habenula. Binding was also prominent in the substantia nigra, zona compacta and ventral tegmental area, molecular layer of the dentate gyrus, presubiculum and cerebral cortex. Cortical laminae III/IV were preferentially labeled; lamina I was also labeled strongly by ^3H -nicotine. Alpha-bungarotoxin binding had an equally discrete distribution pattern. In general, binding was highest in cerebral cortex, hypothalamus, hippocampus, inferior colliculus and in certain brainstem nuclei. In cerebral cortex, laminae I and VI were strongly labeled. Within the hypothalamus, binding was particularly dense in the mammillary body, and in the supraoptic, supraoptic, paraventricular and posterior nuclei. The CA4 area of the hippocampus was also densely labeled. Regions of pons and medulla with appreciable BTX binding included locus coeruleus, dorsal tegmental nucleus. The thalamus and striatum appeared devoid of binding and there was little or no labeling in substantia nigra or ventral tegmental area. Various lines of evidence suggest that the high-affinity agonist binding sites in brain correspond to nicotinic cholinergic receptors similar to those found at autonomic ganglia; BTX binding sites may also serve as receptors for nicotine, and are possibly related to neuromuscular nicotinic cholinergic receptors.

Lesions of the habenula significantly decreased the binding of ^3H -nicotine in most sub-nuclei of the interpeduncular nucleus. These findings suggest that nicotinic receptors may be localized on the terminals of the habenula-peduncular pathway. Lesions of the dorsal tegmental nucleus had no appreciable effects on the binding of ^3H -nicotine to the anteroventral thalamus which receives most of its cholinergic innervation from the lesioned area.

Single cell recording studies revealed that systemically administered (s.c) nicotine increased the spontaneous activity of zona compacta neurons in the substantia nigra. These effects were blocked by nicotinic blockers that penetrate the blood-brain barrier but not peripherally acting antagonists. Zona reticulata neurons were also excited by systemically administered nicotine. This effect however may not be pharmacologically specific but may instead be related to the actions of nicotine on peripheral nociceptors. Interestingly, the zona reticulata neurons that were excited by nicotine were also excited by noxious input.

Injections of nicotine were found to produce mild rotational behavior ipsilateral to the 6-OHDA lesion of the substantia nigra. Furthermore, 6-OHDA lesions of the medial forebrain bundle decreased binding of ^3H -nicotine in the zona compacta of the substantia nigra. All of these findings suggest that nicotine exerts modest effects on the dopaminergic nigrostriatal system through the zona compacta of the substantia nigra.

Intraventricular PCP was found to produce depression of locomotor activity during the first 30 minutes post injection. Only the two highest doses, however,

enhanced locomotor activity during the next 30-45 minutes. Injections into the ventral thalamus, caudate nucleus and ventral tegmental area had no effect on locomotor output. Injections into the nucleus accumbens, on the other hand, produced an immediate and significant elevation in both horizontal and vertical components which seemed to persist over the course of an hour. This effect was reversed by pretreatment with 0.25 mg/kg haloperidol i.p., suggesting dopaminergic involvement. Injections into the periaqueductal gray matter and mesencephalic reticular formation had little effect on horizontal activity while significantly depressing the vertical component during the first 15-30 minutes. These findings suggest that PCP exerts its differential effects on locomotor behavior through different brain structures.

Calcitonin was found to induce patent analgesic effects following injections into the periaqueductal gray matter. All levels of this structure appeared to be equally responsive. Injections of calcitonin into the ventral thalamus, reticular formation and hypothalamus were ineffective. Injections into the pontine reticular formation were also effective. Calcitonin analgesia appears to involve a number of brain structures.

Rats in the VTA group self-stimulated at rates of 65-90 responses/min, while rats in the SNC group at rates of 40-60 responses/min. VTA rats were also more active and behaviorally aroused than SNC rats during the experimental procedure. LCGU was measured in the VTA and SNC, as well as in the terminal fields of each system, both ipsilateral and contralateral to the electrode site. Despite differences in response rates and behavior, similar increases in LCGU at the sites of stimulation and in the ascending and descending fiber pathways were found in both groups with some evidence of topographic organization. Metabolic activation in the SNC rats extended rostrally in the medial forebrain bundle in an area located dorsolateral to the corresponding area in VTA rats. Divergent patterns of LCGU alteration were evident in the terminal fields of the SNC and VTA. For example, in the caudate, a region more heavily innervated by the SNC than the VTA, extensive changes were seen in the SNC rats, but not in the VTA group; whereas in the septum which receives projections from the VTA, but not the SNC, changes were found in VTA rats, but not in SNC rats. In contrast there were several regions including the nucleus accumbens and prefrontal cortex in which similar changes in LCGU were found in both groups. This convergence of metabolic activation despite differences of anatomical innervation suggests a significant role for these regions in mediation of goal-oriented self-stimulation behavior.

Animals that were allowed to self-stimulate (ICSS) and animals given experimenter-administered stimulation (EAS) showed a similar pattern of metabolic activation, as assessed by changes in LCGU, at the stimulation site and in the direct rostral and caudal projections. There was an intense increase in LCGU at the stimulation site in the SNC which continued rostrally within the dorsolateral hypothalamic preoptic area. Caudal to the stimulation site there were bilateral LCGU increases in the projection fibers extending through the pontine gray. In thalamic sensory-motor nuclei, as well as in sensory motor neocortex and cerebellum, LCGU was bilaterally increased in both the ICSS and EAS relative to the NS group, reflecting the general behavioral activation of stimulated rats.

The pattern of LCGU changes within cortical and striatal terminal fields and major striatal efferent pathways was strikingly different in the ICSS and EAS groups. Particularly noteworthy were bilateral increases in LCGU in the medial prefrontal cortex, entorhinal cortex, caudate nucleus, nucleus accumbens and the ventral pallidus in the ICSS group, which were not activated in the EAS group. These data indicate that the distribution of changes in LCGU found in ICSS rats is the result of the goal-oriented nature of their behavior and not simply the consequence of electrical stimulation to the substantia nigra.

The distribution of alterations in metabolic activity in the high current and low current+AMP groups was largely as previously described. Rates of LCGU at the stimulation site and pathway were much lower in the low current+AMP group than in the high current group. In contrast, rates of LCGU in a number of the projection areas of the VTA were equivalent in the two groups, including the nucleus accumbens, the medial prefrontal cortex, the basolateral amygdala, and the locus coeruleus. In the olfactory tubercle and the sulcal cortex, LCGU was higher in the low current+AMP group than in the high current group. Rates of LCGU in the AMP alone group indicated that these effects were in no case due to the amphetamine administration alone.

These findings indicate that equivalent response rates for reinforcing stimulation, whether arising from pharmacological manipulation or varying current amplitude, yield strikingly similar patterns of local cerebral glucose utilization.

Following experimenter administered stimulation to the substantia nigra, regional analyses of caudate/putamen (CP) LCGU rates indicate that the dorsolateral "shoulder" of the CP has consistently higher LCGU rates than other regions of similar size. 6-OHDA pretreatment (3 weeks) reduced LCGU activity throughout the ipsilateral CP over the entire rostrocaudal axis. In unstimulated rats the dorsolateral CP LCGU rates were identical between the electrode implanted and the control side. Nigral stimulation caused a 10 umole/100g/minute LCGU elevation in a restricted region of the rostrocaudal axis. This elevation was restricted to the CP's dorsolateral boundary. Spatially restricted and modest in magnitude, this localized elevation in CP LCGU is associated with much larger metabolic changes in nuclei receiving output projections from the CP (globus pallidus and entopeduncular nuclei). These disproportionate changes may reflect the amplification function of the caudate/putamen in the basal ganglia.

Stimulation of the arcuate nucleus produced profound analgesia in the rat. This analgesic effect was antagonized by 5 mg/kg of naloxone suggesting involvement of endorphins in this response. Stimulation of this area was also found to decrease binding of ^3H -diprenorphine to various terminal regions of the beta-endorphin system following i.v. injections. Stimulation of the periaqueductal gray matter also produced analgesia. This effect, however, was not antagonized by naloxone and was not accompanied by alterations in the binding of ^3H -diprenorphine following i.v. administration. These findings suggest that the analgesic effects of arcuate stimulation are due to the release of endorphins in the terminal areas of this system while the analgesic effects of periaqueductal gray stimulation do not involve the release of endorphins.

Significance to Biomedical Research and the Program of the Institute

Nicotine is one of the most abused substances in society. Understanding its neuronal mechanisms of action will aid in understanding the abuse properties of this substance. It has been suggested that the cholinergic system also plays an important role in mental disorders. It is therefore necessary to understand the functions of this system in brain and to analyze its interactive effects with other neurotransmitter systems such as dopamine. Phencyclidine is also an increasingly abused substance. Furthermore, phencyclidine produces effects in man very similar to some of the primary symptoms of schizophrenia. For these reasons it is valuable to understand the mechanisms of action of this class of compounds. Since reward mechanisms play such an important role in behavior and probably underlie a number of emotional processes, it is imperative to understand the circuitry underlying reward and reinforcement. The self-stimulation paradigm has been useful in the analysis of reward. Since opiates are among the most potent psychotomimetic and euphorogenic compounds, it is of special significance that these exert endogenous compounds in brain which are apparently mimicked by opiates. Defining under what physiological events these endogenous substances are released is an important research issue.

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Clarke, P.B.S., Pert, C.B., and Pert, A.: Autoradiographic distribution of nicotine receptors in rat brain. Brain Res., 1984, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00400-02 BPB

PERIOD COVERED

October 1, 1983 - September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Protein Phosphorylation in Brain

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Jitendra Patel	Unit on Neurochemistry	BPB NIMH
Paul J. Marangos	Chief, Unit on Neurochemistry	BPB NIMH
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COOPERATING UNITS (if any)

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National Institute of Neurological and Communicative Disorders and Stroke

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TOTAL MAN-YEARS:

1.4

PROFESSIONAL:

1.2

OTHER:

0.2

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- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The study of protein phosphorylation is expected to enhance our understanding of biochemical events linking the neurotransmitter receptor to the physiological responses it elicits. During the past year we have extended our research on a unique brain phosphoprotein we had described previously, whose phosphorylation is inhibited by S-100 and by calmodulin. This protein, called S100 modulated phospho-protein (SMP), is found to be present in both brain supernatant and membranes. The membrane SMP was indistinguishable from the supernatant SMP. A brain-extracted growth factor called neurite elongating factor (NEF) was found apparently to convert SMP from the 87k molecular weight form to a 90k form. This property of NEF was further characterized.

Evidence was obtained to suggest that some hormone receptor sensitivity may be regulated by a mechanism involving protein phosphorylation by C-Kinase. Further elucidation of this process is in progress.

Our previous observation regarding the changes of protein phosphorylation associated with kindling were extended. Three phosphoproteins were identified of which the phosphorylation was found to be consistently enhanced in amygdala kindled animals. Such a change in protein phosphorylation was found to be localized to amygdala and could not be detected in prekindled animals.

Project Description:Objectives:

A fundamental aspect of the molecular mechanism of brain function involves the interaction of neurotransmitter with its specific receptor, eliciting a chain of intracellular events which culminates in a biological response. This response includes changes in permeability of the neuronal membrane to various ions, changes in neurotransmitter synthesis, and/or changes in neurotransmitter release. A common early response consequent to the ligand-receptor interaction is the generation of the second messenger. Cyclic AMP, cyclic GMP and calcium are the three major second messengers utilized by mammalian cells. Evidence suggests that most if not all of the second messenger actions on neuronal function of cyclic AMP and cyclic GMP, and many of the second messenger actions of calcium, are accomplished through activation of specific protein kinases. Brain contains two forms of cyclic AMP-dependent protein kinase, a single form of cyclic GMP-dependent protein kinase, one form of calcium-dependent kinase, which also requires phosphatidylserine and diacylglycerol for activity, and four forms of calcium-dependent kinase requiring calmodulin for activity. A number of receptors (e.g., insulin receptor) have been recently identified to possess intrinsic kinase activity, thus omitting for certain responses the requirement for the second messenger.

The main theme of our studies in protein phosphorylation has been to obtain a more precise understanding of the role protein phosphorylation plays in synaptic function. In this task we have assumed the following approach.

1. Characterization of neuron-specific phosphoproteins.
2. Investigation of possible changes in protein phosphorylation in animal models where a dramatic change in synaptic efficacy is suspected.
3. Investigation of the possible involvement of protein phosphorylation in the regulation of receptor sensitivity.

Methods Employed: 1) Gel electrophoresis, single and double dimensions; 2) autoradiography; 3) protein purification techniques; 4) tissue culture; 5) kindling paradigm.

Major Findings:

Previously we reported a unique protein of which the phosphorylation could be inhibited by both S-100 protein, which is a brain-specific calcium binding protein, and calmodulin. Because of its sensitivity to S-100, we termed this protein S-100 modulated phosphoprotein (SMP). Earlier work dealt mainly with the SMP in brain supernatant and so SMP in brain membranes was further characterized. The membrane SMP was found to have properties very similar to those found with cytosolic SMP. Interestingly, the membrane SMP remained attached to the membrane even after repeated washing, but dissociated from the membrane when phosphorylated. This led us to examine the partition of SMP between membrane and supernatant more closely.

If the brain supernatant was prepared from brain homogenized in the presence of high concentration of Ca^{2+} chelators such as EGTA, the phosphorylation of SMP was significantly decreased or was completely absent in the presence of calcium.

Addition of phosphatidylserine and diacylglycerol (PTS-DAG) to the phosphorylation reaction mixture stimulated the phosphorylation of SMP to levels comparable to those obtained with brain supernatant prepared with EGTA. Phorbol 12-myristate 13 acetate (PMA) was also found to be a potent stimulator of SMP phosphorylation. This has led us to the belief that SMP is normally phosphorylated by C-Kinase (or phosphatidylserine-dependent protein kinase) since it is known that this kinase can be activated by PTS-DAG and PMA. The phosphorylation of SMP previously observed in supernatant prepared without EGTA was likely due to the conversion of C-Kinase to a phosphatidylserine independent form during tissue fractionation. Such a conversion, catalyzed by a specific protease, has recently been reported. By performing limited proteolysis of phosphorylated SMP extracted from polyacrylamide gels, we have been able to demonstrate that SMP is phosphorylated at two distinct sites. The nature of the amino acid phosphorylated in SMP is currently being investigated.

The mechanism by which the S-100 protein and calmodulin inhibit the phosphorylation of SMP was investigated. It was found that neither calmodulin nor S-100 protein-mediated inhibition of SMP phosphorylation could be antagonized by increasing either the PTS-DAG or Ca^{2+} concentrations; suggesting that the mechanism of inhibition does not involve sequestration of either of these agents by calmodulin or S-100. Another likely possibility is that S-100 and calmodulin inhibited SMP phosphorylation by stimulating its dephosphorylation by phosphoprotein phosphatase. This possibility was examined using ^{35}S -thio adenosine triphosphate (^{35}S -thio ATP). It was first demonstrated that SMP became resistant to dephosphorylation when phosphorylated with ^{35}S -thio ATP unlike when phosphorylated with the ^{32}P -adenosine triphosphate (^{32}P -ATP). However, SMP phosphorylated by both ^{35}S -thio ATP and ^{32}P -ATP was susceptible to inhibition by S-100 and by calmodulin, suggesting that the inhibition by these agents is not due to stimulation of phosphatase. It is possible that calmodulin and S-100 mediate their inhibitory effect by interacting with SMP directly. This possibility is currently being investigated.

The following work was performed in collaboration with Dr. D. Kligman. Dr. Kligman has isolated a factor from mammalian brain capable of stimulating the growth of neurites in cultured CNS neurons which is called neurite elongating factor (NEF). The mechanism of action of NEF was not known, but for a number of reasons phosphorylation was suspected to be involved. We found that NEF could markedly stimulate the phosphorylation of a single membrane protein of approximate molecular weight of 90,000 (90K). The stimulation of 90K phosphorylation by NEF was found to be rapid, occurring within seconds after the addition of NEF to the brain membrane. The maximal stimulation was obtainable after 1 min. incubation at 30°C. The stimulation of the 90K protein phosphorylation could occur in the absence of calcium, but was found to be greatly enhanced in the presence of calcium. The NEF stimulation was dose-dependent with half maximal stimulation achieved at 20 units of NEF, where 1 unit is that amount of NEF required to obtain half maximal stimulation of neurite extension.

It was observed that a reciprocal relationship existed between the phosphorylation of the 90K and SMP; when the phosphorylation of 90K was stimulated an equivalent decrease in SMP phosphorylation was obtained. Limited proteolysis of the 90K protein generated phosphorylated fragments identical to those obtained from SMP. The pI values of SMP and 90K protein were both approximately 4.5. Phosphorylation of the 90K protein led to its release from membrane in a manner similar to that observed with SMP. The subcellular distribution of SMP and 90K

protein was found to be similar. The S-100 protein and calmodulin also inhibited the phosphorylation of the 90K protein. These and other properties have led us to the belief that the 90K protein and the SMP are the same. Our working hypothesis is that SMP may exist in two forms that can be phosphorylated, each with a molecular weight of 90,000 or 87,000. In the absence of NEF the 90K is converted, perhaps by proteolysis, to the 87K form. NEF is somehow able to inhibit this conversion, permitting its phosphorylation in the 90K form. Experiments to establish the validity of this hypothesis are in progress.

One of the most important properties of the neurotransmitter receptors is the ability of its modulation by agonists and antagonists. For example, chronic exposure to agonist usually causes the receptor to become desensitized to that agonist. The mechanism for such desensitization is not known. The possible involvement of phosphorylation in the regulation of receptor sensitivity was examined in murine leydig tumor cells in collaboration with Dr. Robert Rebois. The leydig cells have adenylate cyclase that is sensitive to lutropin and its analogue human choriotropin (hCG). Binding of hCG to its receptor stimulates cyclic AMP and causes the system to become refractory to further stimulation by hormone. Cyclic AMP is not likely to be involved in the desensitization process, since dibutyryl cyclic AMP cannot mimic the hCG-induced desensitization. We found that PMA could cause the desensitization of the hCG response. PMA-induced desensitization was indistinguishable from hCG-induced desensitization by all of the criteria tested.

Since most, if not all, of the actions of PMA are mediated by C-Kinase, this suggests that the PMA- and hCG-induced desensitization involves phosphorylation of one of the regulatory components of the adenylate cyclase. Treatment of the intact cell or isolated membranes with PMA, hCG or dibutyryl cyclic AMP caused a phosphorylation of many proteins associated with the membrane fraction, but we were unable to distinguish a protein that was phosphorylated by both hCG and PMA but not by dibutyryl cyclic AMP. Recently, by exposing hCG antibodies to solubilized hCG receptors with the hormone bound to it, we have been able to precipitate the hCG receptor. Using such an approach we have been tentatively able to show that hCG receptor does become phosphorylated when exposed to hCG. Work is in progress to establish this.

Amygdala kindling represents a good model for the study of the sensitization process in intact animal. The biochemical changes that are responsible for the acquisition of the kindling behavior are believed to be permanent and for this reason this model is particularly suitable for investigations aimed at determining the role of protein phosphorylation in a specific animal behavior. Previously we have demonstrated that electrical kindling results in an increased phosphorylation of a specific brain membrane protein of molecular weight 45,000. This increase in phosphorylation was found to be bilateral and absent in pre-kindled animals. We have now further analyzed the kindling-associated changes in phosphorylation. We have found that electrical kindling of the left amygdala results in an increase in the phosphorylation of SMP, synapsin and the 35K (previously reported as 45K) protein in left and right amygdala membranes. Pre-kindled animals which do not display Stage 4 or 5 seizures did not display such increases in protein phosphorylation. Other brain areas of the kindled animal, including hippocampus, olfactory lobe and cerebellum, also failed to display any alteration in protein phosphorylation. No significant changes in the phosphorylation of various proteins were observed in hippocampus when kindled with lidocaine. It,

therefore, appears that although the phosphorylation of a number of proteins is altered in amygdala with kindling, these changes are restricted to amygdala and occur only with electrical kindling.

Significance to Biomedical Research and the Program of the Institute: The study of protein phosphorylation is of vital importance in the quest for better understanding how the neurotransmitters mediate their action. Such an understanding is the necessary prerequisite for the appreciation of the biological basis of normal and abnormal behavior.

Proposed course of the project: These studies are expected to continue for the next several years.

Publications:

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NOTICE OF INTRAMURAL RESEARCH PROJECT

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October 1, 1983 - September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Basic and Clinical Studies of Neuronal and Glial Enolases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Paul J. Marangos, Chief, Unit on Neurochemistry, BPB, NIMH
(Other collaborators - see following page)

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TOTAL MAN-YEARS:

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PROFESSIONAL:

0.5

OTHER:

0.9

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 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Our laboratory has played a major role in the discovery and characterization of the neuronal antigen Neuron Specific Enolase (NSE) and its glial counterpart Non-Neuronal Enolase (NNE). We have utilized our specific NSE Antibody to develop a highly sensitive radioimmunoassay and immunocytochemical procedure to do both quantitative and qualitative studies at the basic and clinical level. For the past two years we have focused on clinical studies where we have shown that two neuroendocrine neoplasms, oat cell lung cancer and pediatric neuroblastoma can be effectively monitored by measuring serum NSE levels. Serum NSE levels are highly elevated in both conditions, returning to normal during remission and rising again with relapse. Serum NSE determinations are rapidly becoming routine clinical procedures in both of these neuroendocrine neoplasms. Studies in 30 Alzheimer's disease patients have shown elevated serum NSE levels relative to age-matched controls. Basic studies have focused on the electron microscopic localization of NSE and on cloning of the gene for NSE so that the mechanisms controlling NSE synthesis can be studied.

Other Professional Personnel

J.M. Polak	Senior Lecturer	Royal Med. School, London
A.G.E. Pearse	Professor Emeritus	Royal Med. School, London
S.R. Bloom	Professor	Royal Med. School, London
John Minna	Chief, Med. Oncology Branch	NCI/NNMC
A. Gazdar	Oncologist, Med. Oncology Br.	NCI/NNMC
D. Carney	Oncologist, Med. Oncology Br.	NCI/NNMC
P. Zeltzer	Ped. Onc. Assoc. Professor	Univ. Texas, San Antonio
D. Johnson	Oncologist, Assoc. Prof.	Vanderbilt Medical Sch.
M. Brownstein	Chief, Lab. Cell Biol.	NIMH
S. Vinores	Immunologist, Dept. of Path.	University of Virginia
L. Rubinstein	Chairman, Dept. of Path.	University of Virginia
N. Cutler	Staff Psychiatrist	Lab. of Neurochem. NIA,NIH
R. Prinz	Endocrinologist	Loyola, Chicago
R. Lloyd	Endocrine Surgeon	Univ. of Mich., Ann Arbor
R. Seeger	Pediatric Oncologist	UCLA Medical School
A. Evans	Chief, Pediatric Oncology	Children's Hosp., Phila.
F. Owen	Biochemist	Div. of Psychiatry, MRC

Project Description:

Objectives:

For the past ten years our laboratory has had as its major interest the characterization of proteins unique to nervous tissue. Our work has centered on both neurotransmitter receptors and on soluble proteins in brain. We have characterized two soluble proteins in brain which have turned out to be two isoenzymes of the glycolytic enzyme enolase. One is specific to glial cells and we have named it non-neuronal enolase (NNE). The other is specific to neurons and neuroendocrine cells and we have named this neuron specific enolase or NSE. Both proteins have been extensively characterized, specific antisera raised to each, and highly sensitive and specific radioimmunoassays developed. NSE is a highly useful marker for neurons and neuroendocrine cells and our current studies are now focusing on its clinical utilization. Serum and CSF levels of NSE can be expected to be totally derived from neurons and neuroendocrine cells, therefore, providing a specific index regarding the state of these cells. Our specific focus has been on neoplasms of neurons and neuroendocrine cells and on degenerative neurologic disorders with defined CNS pathology. Studies in these areas (especially the former) have proven to be highly rewarding and the NSE methodology has been rapidly incorporated as a routine clinical procedure in two major neuroendocrine neoplasms.

At the basic level our major objective is to learn more about the biology of NSE. Specifically we are focusing on the developmental switch from NNE to NSE that we first described several years ago. NSE is a marker for neuronal differentiation and we would like to learn more about the genetic mechanisms involved. These studies require the use of recombinant DNA technology which we have sought, and the cloning of the gene responsible for NSE synthesis. Elucidation of the genetic mechanism involved in the initiation of NSE synthesis will provide important insights into the processes which regulate the neuronal phenotype.

Our laboratory has been in large part responsible for the development of the NSE methodology to its current state as a clinically important entity. Further characterization of this system at both the basic and clinical levels is, therefore, a task uniquely suited to our group as well as a scientific responsibility of great interest.

Methods Employed: Radioimmunoassay, immunocytochemistry, clinical procedures such as surgery, blood drawing, etc. Recombinant DNA technology.

Major Findings:

During the past year our recent clinical findings relating to serum NSE levels in oat cell lung cancer and pediatric neuroblastoma have been expanded and further characterized. At the basic level we have completed several immunocytochemical studies on NSE in human lung tissue which have shown that both the nerves and the neuroendocrine dense core granule-containing cells of the lung can be visualized using our antibody. The results of these studies have been published in Life Sciences and Thorax. We have also investigated the histology of small cell carcinomas and carcinoid tumors of the lung showing that the neuroendocrine small cells stain intensely for NSE while other non-neuroendocrine lung tumors do not stain. This establishes the specificity of NSE immunocytochemistry for small cells as well as the utility of this methodology for tumor typing. This study was published in Histopathology. As relates to serum NSE levels in oat cell lung cancer, we have extended our earlier findings by showing similar elevations in diagnosed patients using a second major patient population supplied by Dr. Johnson at Vanderbilt University. Of particular interest in this study was the fact that serum NSE levels were found to be better than conventional clinical means for predicting relapse. In 60% of the patients, serum NSE levels rose as much as 12 weeks before positive chest x-rays were observed. This should prove to be of critical importance in the treatment of this most deadly cancer. These studies were submitted to Cancer Research.

Our collaboration with the Children's Cancer Study group at UCLA concerning pediatric neuroblastoma patients has continued this year in a productive manner and expanded to include a major collaboration with Dr. Audrey Evans who heads the neuroblastoma division at Children's Hospital in Philadelphia. We have shown that 98% of stage IV neuroblastomas have elevated serum NSE levels and that clinically related E wings tumor patients are normal. This supports our initial finding with the UCLA group and extends it since we also showed in this study that serum NSE levels reflect the clinical course of the illness, i.e., they go down at remission and up at relapse. These studies have been submitted to Cancer. Very recent studies with the UCLA group have shown that the NSE antibody can be highly useful in analyzing bone marrow specimens from neuroblastoma patients. To date about a dozen marrows have been examined and tumor cells can be detected at a level of about 1 in every 100,000 cells. This is extremely important information regarding bone marrow re-implants since they must be free of tumor cells to insure successful treatment of the disease. We, therefore, predict that our antibody will be routinely used for this clinical procedure.

Our work on NSE in pancreatic islet and intestinal carcinoid tumors has continued this year with results showing that these endocrine tumors are rich in NSE,

data that is very useful for tumor typing. Serum NSE levels in these disorders are also elevated but to a much lower degree and frequency as compared to oat cell lung cancer and neuroblastoma. Patients with elevated levels do, however, show decreased levels following surgery. These studies have been published in the journal Surgery.

Our collaborative studies with Dr. Lloyd relating to medullary thyroid carcinoma are continuing with immunocytochemical studies that have clearly established a rich supply of NSE in the endocrine cells of these tumors. As in the islet cell tumors, these tumors show only moderately elevated NSE serum levels which cannot be strictly used for diagnosis. These studies have been published in Cancer.

Our long-standing collaboration with Drs. Polak and Bloom has continued with the further exploration of other APUD cells of the diffuse neuroendocrine system. This year we have shown that APUD cells of the pituitary are rich in NSE with virtually all of the corticotrophs staining without antibody. These studies were published in Neuroendocrinology. Both NSE and S-100 immunostaining has been shown to be highly useful in visualizing the peptide secreting cells of the gut and respiratory tract, with these studies being published in Experientia and Thorax, respectively. NSE has also been described in the peptidergic nerves of the human male genital tract, with these results published in the Journal of Urology. NSE is, therefore, holding up quite nicely as being a general marker for the entire diffuse neuroendocrine nervous system and provides further evidence that these neuroendocrine, peptide secreting cells are close neuronal relatives.

In CNS pathologies we have focused on central brain tumors, CNS trauma and Alzheimer's disease. In the first area we have shown that some of the giant cell astrocytomas contain NSE reactive cells. This is the first time we have seen NSE in a glial cell and suggest that the transformation process for these tumors may involve reversion to a neuronal phenotype or that these cells have a neuronal nature heretofore undescribed. These studies have appeared in Acta Neuropathologica. Similar studies with rat experimental gliomas have also been done and are in press in Cancer Research.

In the area of head trauma we have shown that elevated serum and CSF NSE levels are a consequence of this physical insult. These studies suggest that such emergency room cases might be effectively monitored by NSE levels and that the extent of CNS injury might be assessed by such a procedure. This data has appeared in Clinica Chemica Acta.

We have just completed a study of 30 Alzheimer's disease patients in collaboration with the Institute on Aging which has shown statistically significant elevations of serum NSE levels. The elevations were small and reinforce our belief that the most robust elevations would probably be observed in patients prior to the onset of symptoms. This study was recently submitted for publication in the Lancet. We are also involved with studying human brain tissue samples from Alzheimer's disease, schizophrenia and Parkinson's Disease patients in collaboration with Dr. Frank Owen in England. These studies have thus far shown a significant decrease in NSE only in the temporal cortex. We have completed four brain areas with ten more remaining. These results compliment the clinical data and suggest that

neuronal loss may be quite localized. These studies are quite elegant since the tissue is well preserved and age- and sex-matched controls are used throughout.

Basic studies relating to NSE are being rekindled since there are several questions concerning the physiology of this protein that remain to be answered, the most important of which is what factors regulate the switch from NNE to NSE synthesis in the developing neuron. In an attempt to answer this we have engaged in a collaborative effort with Dr. Brownstein at the NIMH and begun an effort that will eventually lead to the cloning of the NSE gene. We are sequencing NSE and will construct a DNA probe which will be utilized to compliment the DNA coding for NSE. Incorporation of this DNA sequence into the appropriate system will enable us to study what factors turn the gene on and off. Such studies are expected to produce results within the next year after a somewhat slow start due to administrative issues at the Institute.

Electron microscopic studies of NSE localization have shown that in rat and mouse, NSE appears to be concentrated in the receptive area of the neuron, i.e., dendrites and cell bodies, with considerably less present in axons and the synaptic area. Also, certain neuronal cell types such as Purkinje cells have less NSE. These studies have been submitted for publication in the Journal of Histochemistry.

During the past year our laboratory has continued to supply research groups with antisera to NSE. Even though there are now 3 commercial sources for the serum we have supplied over 50 groups with antisera during the past year and will continue to do so as a service to the scientific community.

Significance to Biomedical Research and the Program of the Institute: The basic and clinical relevance of the NSE methodology is rather broad since this protein provides a chemical assay for differentiated neurons and its level in biological fluids is highly correlated with the clinical course of various neuroendocrine, neoplasms, and CNS degenerative diseases. The NSE methodology has in large part been developed at the NIMH and represents a unique contribution of this institution to the biomedical field.

Proposed Course of the Project: This project is expected to continue for several years.

Publications:

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Simpson, S., Vinik, A.I., Marangos, P.J., and Lloyd, R.V.: Immunohistochemical localization of neuron-specific enolase in gastroentero-pancreatic neuroendocrine tumors: Correlation with tissue and serum levels of neuron-specific enolase. Cancer, in press.

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Vinores, S.A., Marangos, P.J., Bonnin, J.M., and Rubinstein, L.J.: Immunoradiometric and immunohistochemical demonstration of neuron-specific enolase in experimental rat gliomas. Cancer Res., in press.

Vinores, S.A., Borrin, J.M., Rubinstein, L.J. and Marangos, P.J.: Immuno-histochemical demonstration of neuron-specific enolase in neoplasms of the central nervous system and other tissues. Arch. Pathol. Lab. Med., in press.

Triche, T.J., Tsokos, M., Linnoila, M., Chandra, R. and Marangos, P.J.: Neuron-specific enolase in the differential diagnosis of neuroblastoma and other small round cell tumors of childhood. In Evans, A. (Ed.): Advances in Neuroblastoma Research. New York, Alan Liss, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 01833-04 BPB

PERIOD COVERED

October 1, 1983 - September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Adenosine Receptors in the CNS

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Paul J. Marangos, Chief, Unit on Neurochemistry, BPB, NIMH

(Other collaborators - see following page)

COOPERATING UNITS (if any) Biological Psychiatry, NIMH, National Institute on Aging, Un. of Cincinnati, National Institute of Arthritis, Digestive, & Kidney Dis., National Institute on Alcohol Abuse & Alcoholism, Nat. Center for Drugs & Biol., Nat. Heart, Bl, Lung.

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Unit on Neurochemistry

INSTITUTE AND LOCATION

NIMH, ADAMHA, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

1.7

PROFESSIONAL:

0.6

OTHER:

1.1

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

During the past year substantial progress has been made in characterizing the adenosine receptor and adenosine uptake site in brain. The major focus has involved the effects of various drugs such as caffeine and carbamazepine on adenosine receptors with results showing that both appear to be antagonists. Ontogenetic and anatomical studies have shown that caffeine obtained thru mothers' milk can modulate the adenosine system in pups and that caffeine has differential effects on adenosine receptor subpopulations. Carbamazepine has been shown to be a competitive inhibitor of the adenosine receptor while having no effect on the adenosine uptake site. The adenosine uptake site has been demonstrated in heart muscle with drugs such as dipyridamole, hexobendine and Dilazep being highly potent inhibitors of binding. We have also completed the autoradiographic localization of adenosine uptake sites in rat brain using [³H]Nitrobenzylthiocinosine binding to slide-mounted tissue slices. The dihydropyridine calcium antagonists such as nimodipine and nifedipine are also good inhibitors of binding. Studies with [³H]Nitrendipine binding have also been performed since it is felt that the adenosine receptor may be coupled to the voltage-dependent calcium channel. These studies have thus far defined the binding site and its ontogenetic profile. The adenosine receptor and uptake site are currently being purified utilizing affinity chromatography. These studies are in the preliminary stages of solubilizing both sites and synthesizing an appropriate adenosine analogue coupled resin. Studies are also in progress concerning the effects of alcohol intoxication and withdrawal on adenosine and benzodiazepine receptors. Peripheral type benzodiazepine receptors increase in response to alcohol while central receptors decrease.

Other Professional Personnel:

Jitendra Patel	Fogarty Visiting Associate	BPB	NIMH
Jean-Philippe Boulenger	Fogarty International Assoc.	BPB	NIMH
Robert M. Post	Chief	BPB	NIMH
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Mitch Finkel	Cardiologist	IR/OD	NHBLI
Randy Patterson	Chief, Exp. Phys. & Pharma.	IR/CB	NHBLI

Project Description:

Objectives

Adenosine is a purine nucleotide whose effects on nervous tissue function are both marked and widespread. It has, however, received, until very recently, only very limited attention by neuroscientists. In addition to its marked effects on nervous tissue, which include depression of neuronal firing and neurotransmitter release, as well as potent modulation of cyclic AMP levels, adenosine is also a potent sedative. It also exerts marked hypotensive effects as observed by lowering of blood pressure. Adenosine, therefore, has a multitude of central and peripheral effects which suggest very strongly that it may function either as a classical neurotransmitter or as a neuromodulator. In many ways the effects of adenosine agonists resemble those of other CNS depressants such as GABA and the benzodiazepines.

Characterization of the adenosine system in brain will very likely provide basic insights into the mechanisms regulating CNS activity and lead to the development of new psychotherapeutic agents with sedative or anxiolytic properties. More importantly, the characterization of this system will lead to an understanding of the physiology of this regulatory purine.

Our approach has over the past several years involved the study of the adenosine receptor since it is thought that virtually all adenosine effects on nervous tissue are mediated by specific cell surface receptors. We have characterized adenosine receptors using the metabolically stable adenosine analogues [³H]cyclohexyladenosine ([³H]CHA) and [³H]Diphenylxanthine, ([³H]DPX). For studies of the brain adenosine uptake site we have pioneered the use of [³H]Nitrobenzylthioinosine ([³H]NBI).

Methods Employed: Receptor binding assays, autoradiography brain dissection, affinity chromatography.

Major Findings

During the past several years we have characterized the adenosine receptor in rat, mouse and human brain and the uptake site in rat brain. We have also defined some of the properties of the voltage dependent calcium channel in rat brain using [^3H]Nitrendipine ([^3H] binding). This past year we have developed our studies in several areas which include: 1) the effects of caffeine on adenosine receptors, 2) the discrimination of adenosine receptors and uptake site, 3) carbamazepine and adenosine receptors, 4) the solubilization and purification of adenosine receptors and uptake sites and 5) the study of alcohol's effect on adenosine and benzodiazepine receptors.

Our studies relating to caffeine have shown that chronic caffeine not only upregulates adenosine receptors (reported last year), but does so in a region specific manner. Adenosine receptors in cerebellum and brain stem are the most markedly affected by caffeine administration while hippocampal and cortical receptors are affected less. We have also done some developmental studies relating to chronic caffeine and its effect on the ontogenetic profile of adenosine receptors in brain. These studies have shown that the caffeine obtained thru mother's milk (material dose equivalent to 4 cups of coffee per day) is sufficient to significantly increase the number of brain adenosine receptors. This is the first demonstration of such a phenomenon. It was also noteworthy that the adenosine uptake site remained unchanged during this treatment as did the benzodiazepine receptor. These studies have just appeared in Life Sciences.

We have also shown that chronic social stress induced by crowding (25 mice per cage) leads to an upregulation of central adenosine receptors. Physical stress in the form of inescapable electric shock did not cause alterations in adenosine receptors. Caffeine did not augment the stress-induced changes in adenosine receptors. This represents the first demonstration of stress-induced alterations in adenosine receptors and has just been submitted for publication in the European Journal of Pharmacology.

In an effort to further characterize the adenosine uptake site we have provided additional information regarding its distinct nature from the receptor. The uptake site has a different distribution as determined by autoradiography, is not modulated by caffeine, is present in the heart (receptor not present by current methods), and has a totally distinct pharmacology compared to the receptor. These data have recently been presented and are in press in the Journal of Receptor Research. Cardiologists are quite interested in these findings since the NBI binding assay in heart tissue can now be used to screen for new antihypertensives.

We have also completed the autoradiographic localization of adenosine uptake sites in rat brain and shown that the distribution of the uptake site is somewhat different from that of the adenosine receptor (done last year). Some brain areas are high in both sites whereas other areas are relatively rich in only one or the other site. It is possible that those brain areas rich in both the receptor and uptake site are those with active adenosinergic function. The autoradiographic data have just been submitted for publication in the Journal of Neuroscience.

Further studies on the adenosine uptake site have revealed that [^3H]NBI binds very nicely to dog and human heart ventricle membranes. A high affinity saturable site has been characterized with binding properties similar to those seen in brain. The clinically useful vasodilators dipyridamole, hexobendine and dilazep are extremely potent (10^{-9}) competitive inhibitors of binding, indicating that they are acting at this site. We have also shown that the calcium antagonists such as nimodipine and nifedipine inhibit [^3H]NBI binding at 10^{-8}M and 10^{-6}M , respectively, while the non-dihydropyridine calcium antagonists such as verapamil and diltiazem are ineffective. The inhibition by calcium antagonists is non-competitive, suggesting that these agents are acting at a site coupled to the adenosine uptake site. These studies have recently been accepted for publication in Life Sciences.

Additional studies on calcium antagonist binding sites are of interest to our group since the relationship of the calcium channel to the adenosine receptor and uptake site is important to understanding the adenosine system. In this regard we have completed a developmental study of the calcium antagonist binding site in chick heart and brain. The results show that the binding site is present much earlier in heart than in brain with a rather gradual increase in brain that parallels the time course of neuronal differentiation. In heart the site appears at a time consistent with the appearance of calcium fluxes. In brain the developmental profile is similar to that observed for the adenosine receptor (done last year). These studies have appeared in the Journal of Neurochemistry. Future studies in this area will focus on what factors affect the development of adenosine receptors and calcium channels in cultured neurons and cultured heart cells.

In an effort to determine what currently used psychoactive drugs interact with the adenosine system, we have found that carbamazepine is a potent inhibitor of both [^3H]DPX and [^3H]CHA binding to brain membranes. The respective potencies are 10^{-6}M and 10^{-5}M which makes them quite physiologic, since therapeutic brain levels of carbamazepine are in the order of 10^{-4}M . The inhibition is competitive and is apparently not correlated with the anticonvulsant potency of a series of carbamazepine analogues. These studies have appeared in the European Journal of Pharmacology. Further studies where carbamazepine is administered in the diet over a period of weeks have shown that adenosine receptors are upregulated in a manner similar to that observed for caffeine. This suggests that carbamazepine is acting as an adenosine antagonist. We are also in the process of obtaining [^3H] carbamazepine and will attempt to demonstrate directly whether a binding site exists for this drug and if so whether or not it is physiologically relevant.

We have also recently initiated a series of studies in collaboration with Drs. Majchrowicz and Tamborska at NIA concerning the effect of alcohol intoxication and withdrawal on adenosine, benzodiazepine and calcium antagonist binding sites. To date we have seen a significant and large (40%) increase in [^3H]R05-4864 binding in several brain areas in both the intoxicated and the withdrawn animals. These changes in peripheral type benzodiazepine receptors are accompanied by apparently opposite changes (decrease) in the central type receptor as reflected by [^3H]B-Carboline ethyl ester. No significant changes were observed in the adenosine receptor or calcium antagonist binding site.

A major effort has been started in our laboratory directed at purifying the adenosine receptor in collaboration with Dr. Daly at NIH. These studies involve

solubilization of the receptor, coupling of an adenosine derivative to sepharose and affinity chromatography. Our goal in these studies is to separate the receptor and uptake site and raise antibodies to each. This will make possible the precise immunocytochemical localization of each entity and enable us to address questions such as whether the respective sites are pre- or post-synaptic.

Significance to Biomedical Research and the Program of the Institute: It is becoming increasingly clear that adenosine is a major neuromodulator and that this system probably plays a major role in regulating CNS function. Increased understanding of this endogenous regulator will provide key insights into brain function and make pharmacologic intervention possible.

Proposed Course of the Project: These studies are expected to continue for several years.

Publications

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED

October 1, 1983 - September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Heritable Characteristics of Cation Transport in Primary Affective Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J. I. Nurnberger, Jr. Medical Officer BPB, NIMH

OTHERS: E. S. Gershon Chief, Section on
Psychogenetics BPB, NIMH

W. H. Berrettini Staff Psychiatrist BPB, NIMH

D. C. Jimerson Staff Psychiatrist BPB, NIMH

Sue Simmons-Ailing Clinical Nurse Expert CC, NIMH

COOPERATING UNITS (if any)

Clinical Center, National Institutes of Health

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Section on Psychogenetics

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project is now terminated. A related study was recently begun on sodium-potassium stimulated ATPase, looking at enzyme levels in relation to lipid composition of the red cell membrane, and protein composition of the membrane by two dimensional gel electrophoresis. This study will be subsumed under the project "Genetic-Biological Studies of Affective Illness (Z01 MH 00085-10 BP)."

Publication:

Nurnberger, J.I., Jr., Pandey, G., Gershon, E.S., and Davis, J.M.:
Lithium ratio in psychiatric patients: A caveat. Psychiatry Res.
9: 201-206, 1983.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00084-10 BP

PERIOD COVERED

October 1, 1983 - September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genetic-Biologic Studies of Psychiatric Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: E. Gershon Chief, Section on Psychogenetics BPB, NIMH

OTHERS: L. DeLisi Medical Staff Fellow BPB, NIMH
 D. Pickar Chief, Section on Clinical Studies NSB, NIMH
 L. Goldin Senior Staff Fellow BPB, NIMH
 D. Pauls Asst. Professor Yale Univ.
 K. Kidd Professor Yale Univ.
 N. Cox Postdoctoral Fellow Washington Univ.

COOPERATING UNITS (if any)

Yale University, University of Miami, Wayne State University, Michigan University, Washington University, NSB, AP, LCS, CNB, NIMH, St. Elizabeth's Hospital

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INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

6.25

PROFESSIONAL:

3.25

OTHER:

3.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Family studies and genetic linkage marker studies are proceeding. In childhood and adolescence, offspring of bipolar patients did not show increased frequency of affective disorders as compared with offspring of controls, although they do in adulthood. A schizophrenia family study is underway. In a large Alzheimer's pedigree, new polymorphisms have been identified, and confirmed by Mendelian transmission in the pedigree. A new linkage of a fibroblast polymorphism to a serum polymorphism was identified.

Muscarinic receptors on fibroblasts were studied in additional ill and well relatives of affective disorders patients, with ill relatives showing higher density than well relatives. This may be a genetic marker of vulnerability for affective disorders.

The molecular genetics laboratory is proceeding with screening for polymorphisms of genes for various neuropeptides. The brain hexokinase gene was identified from a cDNA library.

OTHER INVESTIGATORS

C.R. Merrill	Chief, Section on Biochemical Genetics	BPB NIMH
N.S. Nadi	Senior Staff Fellow	BPB NIMH
J. Egeland	Professor	Univ. of Miami
J. Baumgold	Research Chemist	BPB NIMH
N. Sitaram	Professor	Wayne State Univ.
S. Detera-Wadleigh	Senior Staff Fellow	BPB NIMH
J.E. Wilson	Professor	Michigan State
F. Karoum	Medical Officer	St. Elizabeth's Hospital, NIMH
R. Wyatt	Chief	AP NIMH
M. Linnoila	Clinical Director	LCS NIAAD
D. Murphy	Chief	CNB NIMH
W. Berrettini	Staff Psychiatrist	BPB NIMH

1. Family Studies

A. Children of Bipolar Patients and Normal Controls (Dr. Gershon)

A family study of psychiatric diagnoses was performed in 29 children of bipolar patients and 37 children of normal controls, ages 6 to 17. The observed prevalence of depression in childhood is increased when both direct interview of children and interview of parents are performed. There were no differences in frequency of major or minor affective diagnoses between the patient and control groups, but there was an increase of non-specific diagnoses in the patient group. Using DSM-III childhood criteria, 10% of patients' children and 14% of control children had had at least one episode of major depression. This suggests that major depression in children is not familiarly related to adult bipolar major affective disorder. Hypomania (2 cases) and mania (1 case) were found only in children of bipolar patients.

B. "High Risk" Study of Offspring of Bipolar Patients (see Z01 MH 00086-07 BP).

C. Family Study of Schizophrenia (Dr. Gershon, Dr. DeLisi)

We have developed structured diagnostic interview procedures for evaluating subjects for a controlled family study of schizophrenia. These include: the use of The Schedule for Affective Disorders and Schizophrenia (SADS) to make major psychiatric diagnoses using a modification of the Research Diagnostic Criteria, The Structured Interview for the DSM-III Personality Disorders (SIDP), family history checklists, obstetrical-prenatal histories, a premorbid social adjustment scale, and a positive/negative symptom scale. We are in the process of using these in three studies.

i. Consecutive admissions to the NIMH 4E inpatient unit

ii. Patients admitted to the Chestnut Lodge Hospital, Springfield Hospital, and the Psychiatric Institute of Washington, D.C., with an RDC diagnosis of Schizophrenia, are designated as the schizophrenic probands. Age and

gender matched volunteers from the surrounding community who have no psychiatric diagnosis in the SADS and SIDP are serving as the control group. We are now in the process of interviewing all first degree relatives of each proband.

iii. Multiplex families (families with more than one schizophrenic in a sibship) are being recruited for biologic investigations. Potential biologic markers are tested in families where more than one first degree relative has the diagnosis of schizophrenia in order to determine whether any of these markers is present in these families and whether they segregate with the illness. These include: structural alterations as seen on CT scans, such as ventricular size, cerebellar and cortical atrophy; phenylethylamine and phenylacetic acid production; and DNA polymorphisms using probes for neuropeptides and other markers. The DNA polymorphisms will be studied as association and linkage markers.

D. Alzheimer's Disease Pedigree Linkage Markers

Sixteen, six and three two-dimensional polymorphisms of fibroblasts, serum, and erythrocytes, respectively, were identified in a population sample and in over 70 persons from one large multigenerational family with Alzheimer's disease. These polymorphisms are identified as charge variants, with heterozygotes showing a gene dosage effect. Five of the fibroblast polymorphisms were shown to be identical to loci previously described in lymphocytes. Three known serum loci (alpha-1 antitrypsin, group specific component, and alpha-2 HS glycoprotein) had previously been found by two-dimensional electrophoresis and were also identified here. All variants could be confirmed by Mendelian transmission in the large family, although some were too rare to be evaluated. Four other serum polymorphisms (PGM1, PGM3, GL01, and ESD) were typed. Lod scores were calculated using the program LIPED to determine linkage relationships among known and unknown markers. One fibroblast polymorphism (NIMH-26) was found to be identical to PGM3, and one erythrocyte polymorphism (RBC-1) was identical to GL01. One fibroblast polymorphism showed evidence for linkage to a serum polymorphism (lod score=2.8 at $\theta=0\%$). There are 34 remaining unidentified loci among lymphocytes, fibroblasts, erythrocytes, and serum. While some of these are likely to be identical to other known protein charge variants, this technique adds new loci that can be used for linkage and mapping studies, with the advantage that many polymorphisms can be identified on a single gel.

The fibroblast, serum, and erythrocyte polymorphisms were also tested for linkage to Alzheimer's disease. No linkage could be detected. However, this analysis has very low power because only 4 affected individuals were sampled, and many younger individuals had not yet entered the age of risk.

E. Mathematical Simulation Study of Analytic Methods (Dr. Goldin)

Simulation studies were continued. The detection of a major locus affecting a common, dichotomous trait was compared by two different

methods of analyses--the major locus model using the computer program, GENPED, and the mixed model using the computer program, POINTER. The two procedures gave nearly equivalent results with respect to major locus detection. Neither procedure could consistently detect a major locus when the heterozygote penetrance was low or intermediate (5-15%). The estimates of the parameters were accurate if the population prevalence was assumed to be known. In the cases where a major locus could not be detected by segregation analysis, the locus could be inferred if a closely linked marker locus was present. These results give us some overall indication of the limits of segregation and linkage analysis.

We have also analyzed the power of high-risk studies (where offspring of patients are compared with offspring of controls). For a given power (one minus the probability of type II error), the required sample size can be determined from the mode of inheritance of the biological traits, the relationship of the trait to illness, and the degree of overlap between the patients and control distributions.

2. Clinical and Basic Studies of the Muscarinic Receptor

A. Cholinergic receptor on fibroblasts:

Muscarinic cholinergic receptor density was studied by Dr. N. S. Nadi in a larger number of patients, first-degree ill relatives, minor illness, and normal relatives. The results (including previously reported numbers) are as follows:

	QNB B_{max} fmol/mg protein
	Mean \pm S.D.
Patients (n=18)	335.1 \pm 58.3
Relatives (n=13) (major affective)	307.3 \pm 50.1
Relatives (n=5) (minor disorders)	291.3 \pm 435
Relatives (n=5) (normal)	189.7 \pm 16.7
Unrelated controls (n=12)	227.8 \pm 47.3

Observations in two pedigree fragments have shown that the well relatives fall below a cutoff value of 225 fmol/mg proteins and the ill relatives are above this value. In an Amish pedigree the values did not follow this trend. This may be due to the Amish showing a different subgroup of the illness. Taken together, these data suggest muscarinic receptor density is a genetic vulnerability marker in a substantial proportion of affective illness pedigrees.

B. Purification of Muscarinic Receptor Protein

Substantial progress has been made in purifying the brain muscarinic receptor protein. Details on the progress of this work can be found in the Annual Report of the Laboratory of Neurobiology.

C. REM Induction by Arecoline

Sensitivity of patients, relatives and controls to the muscarinic agonist arecoline is being studied; see Z01 MH 00085-08 BP.

Preliminary results confirm, in a new series of patients and controls, increased sensitivity to cholinergic REM induction as a state-independent trait marker in affective illness. We have previously demonstrated that this is apparently a heritable trait, and Dr. Sitaram has recently demonstrated association of this trait with illness in relatives of patients. These data suggest that this measure of cholinergic sensitivity may identify a genetic vulnerability factor in affective illness.

3. Molecular Genetic Studies (Dr. Detera-Wadleigh)

A. Polymorphic DNA Markers in Neuropsychiatric Disorders

Molecular biology has provided a powerful tool toward the discovery of the primary genetic defects in a neurodegenerative disease, Huntington's chorea by J. F. Gusella and colleagues. Dr. Detera-Wadleigh is using this approach to examine the genetic aspects of primary affective disorders and schizophrenia. We are, therefore, undertaking a search for restriction fragment length polymorphisms that may be linked to the gene loci of these neuropsychiatric illnesses. The project involves isolation of genomic DNA from normal individuals, patients and family members of potentially informative individuals. DNA's obtained from either cultured lymphoblasts or whole blood are systematically digested with different restriction enzymes, size fractionated on agarose gels and transferred to nitrocellulose filters by Southern blotting. The DNAs on the filters are hybridized to cDNA probes of interest. Currently, we are using cDNA's that encode certain neuropeptides and brain proteins. Some of these probes have been shown to be polymorphic in samples of the normal population. Cultures of the cDNA clones have been provided to us by collaborators. Identification of a DNA marker will have very important application in clinical care as well as basic studies on the gene defect.

B. Cloning of the Brain Hexokinase Gene

Hexokinase is an important brain enzyme involved in the initial steps of glucose metabolism. Dr. S. Detera-Wadleigh has isolated two specific cDNA clones for hexokinase by screening a λ gt11 cDNA library from one-week-old rat brain. The clones are detected using an antibody to brain hexokinase. This is possible because λ gt11 is an expression vector. Characterization

and structural analysis will be performed in J. E. Wilson's laboratory, at Michigan State University. These clones will be used also in isolating the hexokinase gene. This will then afford the study of gene expression in certain disease states.

4. Other Biologic Studies

A. Phenylethylamine Metabolism (Dr. DeLisi)

Studies have been completed comparing urinary phenylethylamine and phenylacetic acid excretion rates in schizophrenic and affective disorder patients and normal controls. A subgroup of psychotic female patients have been found to have markedly elevated phenylethylamine excretion, exceeding at least three times the normal range. It is unclear, however, how this relates clinically to diagnosis, symptoms, or psychiatric state of the individual at the time of the urine collection, and the significance of the gender difference has not been explored. Nevertheless, one NIMH severely psychotic affective disorder patient was reported to significantly improve with the use of carbidopa, a peripheral decarboxylating agent known to decrease peripheral phenylethylamine production. We are presently investigating the use of this drug in patients who are found to have elevated phenylethylamine production. In addition further studies exploring the pathogenesis of elevated phenylethylamine production, including genetic studies, are in progress.

B. Proteins and Peptides in CSF (Dr. Berrettini)

In continuing outpatient CSF studies of 30 normal volunteers, 25 lithium-treated, and 15 unmedicated euthymic bipolars, we have measured 5 POMC-related peptides (alpha-MSH, beta-endorphin, ACTH, N-Terminal fragment of POMC, and alpha-LPH) in CSF and plasma. The results suggest some subtle abnormalities in the processing of POMC in bipolars. Additionally, we have measured neurotensin, vasopressin, calmodulin, calcitonin, somatostatin, VIP, GABA and monoamines and their metabolites, none of which differentiate bipolar patients from controls. We have studied these CSF samples with 2-dimensional gel electrophoresis, in a search for abnormal proteins; however, no clear abnormalities have been detected in the bipolar group.

C. Immunology (Dr. DeLisi)

Evidence is increasing that suggests that some schizophrenic and depressive patients have deficient functioning of multiple components of the immune system. It is unclear whether the multiple immune abnormalities reported in these patients reflect evidence for a viral etiology to the psychiatric illness, chronic use of neuroleptic or antidepressant medication, abnormal neurotransmitter and neuroendocrine metabolism, or a primary inherited defect in the immune system itself. We have completed some initial studies quantifying functioning of different components of

the immune system in schizophrenics, depressives, and Huntington's Chorea patients and have found subgroups of patients with decreased immunoglobulin production, decreased natural killer cell and macrophage functioning, and increased autoantibody production. We are continuing to explore the significance of these findings in longitudinal and family studies.

5. Basic Studies on Neurotransmitters (Dr. Nadi)

A. Glutamate

Glutamate has been proposed as the transmitter in granule cells, and aspartate as the transmitter in climbing fibers in the cerebellum. Dr. Nadi has found, in rat, that: i. Glutamate and aspartate receptors are differentially distributed in the layers of the cerebellum. ii. The receptors are differentially affected by antigitamatergic compounds, suggesting that different receptor sites for aspartate and glutamate exist in the cerebellum. iii. Destruction of climbing fibers, which are presumably aspartate-containing neurons, alters the ^3H aspartate binding but not glutamate binding, suggesting once again two separate sites. Further studies involving the destruction of granule cells, which contain glutamate, are under way to further study these two receptors.

A peptide with glutamate-like activity was isolated from the cerebellum. Further purification and characterization of this peptide is underway.

B. Effects of Adrenalectomy and Hypophysectomy on Cholinergic Function in Brain

Choline acetyltransferase (CAT) in brain parts from adrenalectomized animals showed a slight but significant increase from 5 to 7 nmol/mg pmol/15 min. The hypothalamus showed a very large decrease from 27 to 9 nmol/mg protein/15 min. There seems to be no direct correlation between the loss of CAT and cholinergic receptors in brain. CAT activity did not show any alteration in any other brain parts.

Hypophysectomy caused an increase in cholinergic binding in the olfactory bulb. This, together with the drop following adrenalectomy suggests that ACTH may be involved in this system.

Significance to Biomedical Research and the Program of the Institute

Successful identification of a marker of genetic vulnerability to affective disorders would lead to identification of the responsible pathophysiological process, and would have clinical applications for prevention and choice of treatment. The muscarinic cholinergic findings in REM sleep and on fibroblasts described in this report satisfy criteria for vulnerability markers, at least in a significant proportion of affective disorders, and it is our hope that these findings will prove replicable in other settings.

Family studies of childhood and adolescence, and development of a high risk study, may contribute to identification of the premorbid state in affective disorders and to testing of genetic vulnerability markers. Family study of schizophrenia may provide vulnerability markers in that disorder, and may also give a delineation of the familial clinical findings related to schizophrenia.

Our identification of new genetic polymorphisms, and analysis of the power of mathematical genetic models, contributes to the general advancement of human genetics, and to the genetics of neuropsychiatric disease.

Two major applications of molecular genetics in clinical neuroscience at this time are the identification of genes for neuropeptides and neuronal proteins, and identification of polymorphisms of these genes. These genes can be used as markers in basic studies of gene expression and in clinical studies relating specific genes to illness.

Proposed Course of the Project

We plan to continue to investigate the biology and genetics of characteristics that may be implicated in the genetics of affective disorders, as described above. Further study of the fibroblast as a clinical neuronal model will proceed. The molecular genetics approach of interindividual differences in neuropeptides and other substance will be pursued. Establishing a library of DNA and living cells from entire pedigrees is a major priority. Study of relatives at risk for affective disorders and schizophrenia will proceed. Mathematical methodology for clinical investigation will continue to be studied.

Publications

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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01MH00085-08 BP

PERIOD COVERED

October 1, 1983 - September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pharmacogenetics of Psychoactive Drugs

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

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C. Gillin Psychiatrist San Diego V.A. Admin.

COOPERATING UNITS (if any)

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LAB/BRANCH

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INSTITUTE AND LOCATION

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TOTAL MAN-YEARS:

0.75

PROFESSIONAL:

0.5

OTHER:

0.25

CHECK APPROPRIATE BOX(ES)

 (a) Human subjects (b) Human tissues (c) Neither (a1) Minors (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Our study of the neurochemical mediation of amphetamine response is continuing with the use of the serotonin receptor blocker metergoline in conjunction with amphetamine. Five persons have participated in pilot studies related to this project.

We have undertaken to use several agents that are reported to cause the release of adreno-corticotrophic hormone (ACTH) because of reported abnormalities of this hormone in affective illness. Thirteen subjects have participated in a pilot study of the opiate antagonist naloxone and five persons in a pilot study of the serotonin precursor tryptophan.

A replication study of cholinergic REM induction is in progress. Four out of six bipolar patients have shown high sensitivity so far, as compared with 1 out 7 controls. This is consistent with our previous findings, and with our hypothesis of increased cholinergic sensitivity associated with vulnerability to affective illness. We plan to extend this study to offspring of patients in the high risk study.

A study of the effect of physostigmine on melatonin is underway. Four persons have participated in a pilot study.

OTHER INVESTIGATORS (CONTINUED)

A. Lewy	Associate Professor	University of Oregon
D. Sack	Chief, Intatient Unit	CPB NIMH
W. Mendelson	Chief, Unit on Sleep Studies	CPB NIMH

Project Description

1. Pharmacologic dissection of amphetamine response

We have previously demonstrated that the behavioral excitation response to amphetamine is blocked by the dopaminergic antagonist haloperidol, and the blood pressure and norepinephrine responses by the beta adrenergic receptor antagonist propranolol. The hormonal responses were not blocked by either of these agents, nor were they blocked by the alpha-adrenergic antagonist thymoxamine.

There is evidence that amphetamine releases serotonin as well as dopamine and norepinephrine, and serotonin may in turn cause the release of pituitary hormones. A study is in progress to determine whether the serotonin receptor blocker metergoline will prevent the rises in cortisol, prolactin, and growth hormone that are caused by amphetamine. A pilot study has been used to examine the hormonal effects of various doses of amphetamine with and without metergoline pretreatment. The combination appears to be well tolerated. Hormonal results are awaited.

2. ACTH stimulation

It is known that cortisol is excessively secreted by depressed patients. This appears to be caused by excess release of ACTH from the pituitary gland. We are examining various neurochemical agents that are known to provoke ACTH release in order to see whether euthymic (well state) bipolar patients are more sensitive to these agents. An abnormal response in well-state patients might be a clue to an underlying genetic vulnerability factor for affective disorder.

The opiate antagonist naloxone and the serotonin precursor tryptophan have been reported to cause release of ACTH. We have performed 31 infusions on 13 subjects with naloxone and 13 infusions on 5 subjects with tryptophan in an effort to find a dose near the threshold for ACTH stimulation. Endocrine results are awaited.

3. Cholinergic REM induction

We have previously reported that well state bipolar patients were more sensitive than normal volunteers to the REM (rapid eye movement sleep) inducing effects of the muscarinic cholinergic agonist arecoline. In this study, a single dose of 0.5 mg arecoline is given intravenously to a sleeping subject 25 minutes after the end of the first REM period. The latency to the beginning of the second REM period is measured. Thus far ten patients and eight normal volunteers have participated. Two patients were not able to

sleep adequately for the study. Of the others, four patients were quick REM inducers and two patients awakened in response to the drug. Of the controls, one was a quick REM inducer and one awakened. Data from the other studies suggests that awakening is analogous to REM induction. We are encouraged by these early data and are planning to pursue this as a possible marker in high risk offspring.

4. Light suppression of melatonin

We have previously reported that bipolar patients are more sensitive than controls to the melatonin inhibiting effects of light. Evidence from the animal literature suggests that the light inhibition effect may be mediated by acetylcholine (probably via nicotinic receptors). We are testing physostigmine to see if it mimics light in reducing melatonin in man. Pilot studies to find a dose of physostigmine that will reduce melatonin without producing unpleasant side effects have begun; four persons have participated in these studies so far.

Significance to Biomedical Research and the Program of the Institute

The cholinergic REM induction studies (together with the data on fibroblast muscarinic receptors from Dr. Nadi's lab) suggest that muscarinic cholinergic supersensitivity may be responsible for genetic vulnerability to affective disorder. If this is the case, it would provide a stimulus for localizing the molecular events that predispose to depression. It also may provide clinically useful genetic vulnerability markers. This is the possibility we are pursuing in our study of high risk offspring. The physostigmine results may tie in with this same vulnerability factor.

The amphetamine studies provide data on neurochemical control of what may be heritable responses to this psychoactive drug in man.

Proposed Course of Project

The course of the cholinergic studies is outlined above. The ACTH stimulation studies will continue with an investigation of the calcium channel blocker diltiazem. We also plan an ACTH infusion study, coupled with a multi-day steroid challenge.

Publications:

Nurnberger, J.I., Jr., Sitaram, N., Gershon, E.S., and Gillin, J.C.: A twin study of cholinergic REM induction. Biol. Psychiatry 18: 1161-1173, 1983.

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Gershon, E.S. and Nurnberger, J.I., Jr.: Is there a cholinergic depression-stress system? Integrative Psychiatry, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01MH00086-08 BP

PERIOD COVERED

October 1, 1983 - September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Outpatient Clinic for Genetic and Pharmacological Studies of Affective Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: J. I. Nurnberger, Jr. Medical Officer BPB, NIMH

OTHERS: E. S. Gershon Chief, Section on Psychogenetics BPB, NIMH
 S. Simmons-Alling Clinical Nurse Expert CC, NIMH
 W. H. Berrettini Staff Psychiatrist BPB, NIMH
 J. Hamovitz Social Worker BPB, NIMH
 E. Hibbs Research Psychologist BPB, NIMH
 E. Maxwell Social Worker BPB, NIMH

COOPERATING UNITS (if any)

Clinical Center Nursing Department, NIH; Catholic University; University of Caen, Clinical Research Institute of Montreal, Montreal, Quebec, LPP, LCS

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Section on Psychogenetics

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

3.5

PROFESSIONAL:

2.0

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Cerebrospinal fluid (CSF) specimens were studied in 30 controls, 15 unmedicated bipolars and 25 lithium-treated bipolars. Further measures of peptides related to pro-opiomelanocortin (POMC) have been done on N-terminal fragment (NTF), adrenocorticotrophic hormone (ACTH) and beta-endorphin (BE), to enable a more complete assessment of the "cascade" of substances originating in POMC. Measurements of calcitonin have also been made. No patient-control differences were noted in the POMC related peptides, or the other neuropeptides investigated.

Lithium treatment was found to decrease plasma and CSF vasoactive intestinal peptide (VIP) but not the other neuropeptides studied. On the lymphocyte, lithium increases affinity of the VIP receptor for its ligand.

Two dimensional electrophoresis has been used to study the spinal fluid proteins from many of these persons. No protein charge abnormalities were associated with bipolar affective disorder.

We have now entered 42 offspring of bipolar parents and 15 controls into our high risk study of affective illness. Thirty-three skin biopsies have been done. The theoretical underpinnings of high risk studies and the number and type of subjects to be studied have been analyzed.

The experimental antidepressant bupropion has been studied in six patients who had not had good response to other agents. Two patients have responded well.

INVESTIGATORS (CONTINUED)

M. Cretien	Laboratory Director,	Clin. Research Inst. of Montreal
P. Gold	Chief, Section on Clinical Neuroendocrinology	BPB, NIMH
M. Harrington	Internationaal Fogarty Fellow	BPB, NIMH
C. Merrill	Medical Officer	BPB, NIMH
W. Kaye	Staff Psychiatrist	LPP, NIMH
H. Gwirtsman	Medical Staff Fellow	LCS, NIMH
D. Pellegrini	Assistant Professor	Catholic University
S. Nadi	Senior Staff Fellow	BPB, NIMH
L. Goldin	Senior Staff Fellow	BPB, NIMH
L. Pons	Professor	University of Caen

Project Description:

We maintain an ongoing treatment clinic for 120 manic-depressive outpatients for the purpose of: 1) identifying potential markers of genetic vulnerability to affective disorder; and 2) studying the course and treatment of affective illness, especially bipolar disorder.

For purposes of comparison, we have 52 normal volunteers and 23 pairs of monozygotic twins.

1. Markers of genetic vulnerability to affective illness

We study primarily "well state" patients to determine those abnormalities that are most likely abiding characteristics of the illness.

CSF Studies

Two years ago, we initiated the first outpatient study of CSF biochemistry. The outpatient methodology has proven to be efficient for screening studies. To date, we have obtained CSF from 35 normal volunteers and 25 lithium-treated euthymic bipolar patients (15 of whom also provided unmedicated samples).

We have studied a dozen neuropeptides by RIA. We also measured GABA, and the monoamines and their metabolites. We did not find any significant group differences. Two effects of lithium were noted on the paired samples. Lithium was found to decrease CSF and plasma vasoactive intestinal peptide (VIP) and increase CSF 5-HIAA.

We measured by RIA 5 fragments of the large precursor prohormone pro-opiomelanocortin (POMC): the N-terminal fragment (NTF); beta-endorphin; beta-lipotropin; alpha-MSH and ACTH. Although there are no absolute differences among groups, several interesting differences appeared in the three patient groups in regard to correlations between the POMC fragments. These differences suggest subtle alterations in POMC processing in euthymic bipolars. Additionally, they suggest that CSF alpha-MSH originates from a source (possibly dorso-lateral hypothalamus) which is independent of the source for other POMC fragments (probably arcuate nucleus).

2-dimensional gel electrophoresis was done on these CSF samples to search for abnormal proteins in the euthymic bipolar group. We studied the qualitative and quantitative characteristics of CSF protein on gels from 30 normal volunteers and 20 lithium treated euthymic bipolars (10 of whom provided unmedicated samples for this study). No group differences or effects of lithium were found.

In addition, we have measured beta-LPH, beta endorphin, VIP and calcitonin in anorexia and bulimia patients. BLPH and beta-endorphin are low in the low-weight anorectics, increase towards normal in short-term weight recovery and decrease again during long-term weight recovery.

We have in progress studies of CSF CRF and Substance P in the euthymic bipolars and controls.

Following our observation that lithium treatment decreases CSF and plasma VIP, we measured lymphocyte VIP receptors in euthymic bipolar patients and controls. Lithium increased the affinity of the receptor for its ligand, providing a possible psychotropic mechanism and a possible explanation for the clinically troubling side effect of diarrhea.

2. High Risk Study of Affective Disorder

We have actively recruited young persons (age 15-25) for this study over the past year. A group of 42 young people with a manic depressive parent has been assembled along with 15 age-matched controls. In each case the young person has been screened with a structured interview (SADS-L), both parents have been interviewed, and one parent has been asked to fill out a childhood symptom inventory. Each young person entering the study has been given a life events assessment. Thirty-three persons have had skin biopsies in order to study muscarinic cholinergic receptors (^3H quinuclidinyl benzilate binding) in cultured fibroblasts (see Genetic Biologic Studies report). We plan to study cholinergic REM induction and light suppression of melatonin. Followup studies are to be conducted yearly over the next ten years.

Dr. Lynn Goldin has made a detailed analysis of the high risk methodology and the restrictions that it puts on biologic variables one may assess. She has found that the sample size needed depends on: 1) the mode of inheritance of the biological trait; 2) the population prevalence of illness; and 3) the difference in the trait distribution between patients and controls.

For example, assume that a trait is measured on a continuous scale and is distributed normally. If this trait were controlled by a polygenic mechanism, then offspring of 1 ill and 1 well parent would have 1/2 of the susceptibility factors. Thus, the magnitude of the patient-control difference would be reduced by 1/2. This would also be the case if the trait were transmitted as a single dominant or additive locus. However, if the transmission were recessive, this difference would be reduced by 3/4. Other more complicated models of transmission can be hypothesized but these represent a reasonable approximation to the problem. Thus, by making some

simple assumptions, we can objectively determine the necessary sample size. For example, in the case of fibroblast muscarinic receptors, patients with affective disorders have a higher density of receptors than do controls, the difference being about 2 standard deviation (s.d.) units. High risk offspring will differ from controls by 1 s.d. if the transmission is polygenic, single locus dominant or single locus additive, and by 0.5 s.d. if transmission is recessive. Assuming a t-test were to be used, only 15 individuals/group would be sufficient to achieve 80% power given the former alternatives, but about 50 individuals/group would be required under the recessive alternative. If offspring of two ill parents can be studied, the power is increased considerably. This is because the expected high risk trait distribution does not deviate much from the patient distribution. Thus, many fewer individuals at risk are needed to demonstrate a susceptibility factor.

3. Studies of the Course and Treatment of Affective Illness

We have begun a study of the antidepressant bupropion, a novel compound that does not have the monoamine reuptake blocking properties or the beta-adrenergic receptor down-regulating properties of the standard agents. Bupropion also has very weak anticholinergic effects avoiding one common source of antidepressant side effects. It also is reported to have anticycling effects in some bipolar patients. Of six patients studied thus far, two have had good to excellent responses. None have had induction of mania or hypomania. One patient with a history of manic episode associated with desipramine has been maintained successfully for 5 months on bupropion.

Fifty-eight patients have participated in a followup study of associative processes in affective illness. We found that lithium enhanced repetition of associative responses in an original study several years ago. Dr. Louis Pons, of the University of Caen, found that lithium enhanced repetition of associative responses in an original study several years ago. He has replicated this finding and has also shown a differentiation between manic depressive patients and panic disorder patients on the basis of associative responses.

Significance to Biomedical Research and the Program of the Institute

The marker studies are aimed at uncovering indicators of genetic vulnerability as discussed in Z01 MH 00085-08 BP. These might then lead us to a better understanding of the etiology of these conditions and enable early identification and monitoring of vulnerable persons.

The high risk study may provide confirmatory evidence for biochemical hypotheses regarding the etiology of manic-depressive illness. If a genetic vulnerability factor or factors can be demonstrated, new treatment strategies may be designed. In addition, clinical tools for the early identification of persons vulnerable to affective illness may be forthcoming. Presently available pharmacologic or psychosocial interventions might then be utilized to prevent the social deterioration that may result from untreated affective disorder.

The antidepressant trials are directed toward the solution of a difficult clinical problem as well as an improved neurochemical understanding of the switch process in bipolar illness.

Proposed Course of Study

CSF variables are to be correlated with clinical variables in the patient population. A study of correlates of lumbar puncture related headache is underway.

The high risk study is to continue as planned with further biologic measurements to be made as indicated above.

The study of bupropion will continue. A new study of biochemical correlates of Tegretol response in bipolar illness is planned.

Publications:

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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00326-11 CN

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical neuropharmacology and psychobiology of depression and mania

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Dennis L. Murphy, M.D., Chief, Clinical Neuropharmacology Branch, NIMH

COOPERATING UNITS (if any)

BPP, CPB, LCS, NIMH; USUHS, VA Med. Cen., Bronx, NY

LAB/BRANCH

Clinical Neuropharmacology Branch

SECTION

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

3.1

PROFESSIONAL:

2.0

OTHER:

1.1

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Two series of studies examining the functional status of brain monoamine systems were completed this year. Using the serotonin agonist fenfluramine in a neuroendocrine challenge paradigm, reduced prolactin responses to fenfluramine were found in a large general sample of depressed patients compared to controls, as well as in a smaller sample of patients carefully age- and sex-matched with controls. To evaluate whether this response difference might represent a presynaptic alteration dependent upon the availability of serotonin for release or a serotonin receptor difference, we are currently using the direct serotonin receptor agonist in the same paradigm in further psychiatric patient groups and controls. We have also completed several sets of studies of the status of brain catecholamine responsivity to the selective α_2 -adrenergic agonist clonidine. Dampened plasma MHPG and heart rate responses but greater plasma cortisol reductions following clonidine were found in depressed patients compared to controls, indicative of alterations in α_2 -adrenergic regulation of catecholamine function in depression. While we have previously found that antidepressant drug treatment modifies these abnormal cardiovascular and MHPG responses to clonidine, no similar changes were observed in platelet α_2 -adrenergic receptors or cyclic AMP responses to norepinephrine, suggesting that this cell may not be as useful a model for evaluating central receptor adaptive events as had been postulated previously.

Other collaborative professional personnel engaged on the project:

B. Roy	Staff Psychiatrist	CNB, NIMH
R. Lake	Staff Psychiatrist	Uniformed Services
L. J. Siever	Staff Psychiatrist	VA Med. Cen., Bronx
E. A. Mueller	Staff Fellow	CNB, NIMH
T. Sunderland	Staff Fellow	CNB, NIMH
R. M. Cohen	Staff Psychiatrist	CNB, NIMH
M. Kafka	Physiologist	NSB, NIMH
T. Uhde	Staff Psychiatrist	BPB, NIMH

Project Description:

Objectives: Individuals with depression, mania, and related disorders with affective components, including individuals with characterologic disorders, those with depression secondary to other psychiatric (e.g. personality disorders) and neurologic disorders (e.g. Parkinson's disease, Alzheimer's dementia), are studied in attempts to understand the psychological and biological mechanisms involved in therapeutic drug effects in these disorders. As individual differences in psychoactive drug responsiveness appear to depend upon many psychological and biological factors, a variety of study approaches are utilized.

Methods Employed:

1. Behavioral and psychological assessment: Pretreatment evaluation of patients requires information from interviews of the patient and family, from psychometric approaches, from direct behavioral observation using various quantitative scales and from patients' self-ratings. The elucidation of individual and patient subgroup differences in drug response depends upon this information obtained by the clinical staff, including psychiatrists, psychologists, social workers and nursing personnel. Subsequent evaluation of drug response depends upon objective behavioral assessment as well as self-rated psychological change as obtained from a number of quantitative scales, several of which have been developed in this Branch.

2. Biological assessment: Pharmacologic challenge tests of central neurotransmitter systems are used to evaluate the functional state of these systems in patients compared to controls, and in patient groups studied before and during antidepressant drug treatment. Neuroendocrine, cardiovascular and behavioral changes are used as endpoints in these studies. Plasma, platelets, urine, and cerebrospinal fluid are collected for the measurement of biogenic amines and their metabolites, enzymes, other chemical variables, and drug levels. Electrophysiologic measurement of sleep and of psychophysiological variables are accomplished in collaborative studies with investigators in other Branches.

Major Findings:

In baseline studies of patients with depression, possible abnormalities in both the central serotonergic and noradrenergic systems were identified. To evaluate the serotonin neurotransmitter pathways, fenfluramine, a serotonin releasing agent, was administered to 18 depressed patients and 10 controls, and placebo was administered to 16 of the depressed patients in a double-blind paradigm. Plasma prolactin levels were measured prior to and for five hours following fenfluramine. Plasma concentrations of prolactin have previously been shown to increase after fenfluramine administration in humans, consistent with evidence from studies in rodents that it and other serotonergic agonists enhance prolactin secretion. Fenfluramine's effect on prolactin, like its anorectic effect is thought to be mediated by increases in central serotonin.

Although the exact mechanisms by which serotonin affects prolactin release are not completely understood most agents enhancing serotonergic activity increase plasma prolactin concentrations, and a wide variety of serotonin antagonists prevent such prolactin responses. In our study, fenfluramine produced a significant increase in prolactin in both patients and controls. However, the prolactin response to fenfluramine whether measured as an absolute increase or percent increase from baseline was significantly less in depressed patients than controls. This difference remained equally statistically significant when age-and-sex-matched pairs of depressed patients and controls were compared. These results suggest that the central serotonergic system may be less responsive in depressed patients than controls.

To evaluate noradrenergic system responsivity in depressed patients, plasma 3-methoxy-4-hydroxyphenylglycol (MHPG), plasma norepinephrine, blood pressure, and heart rate responses to the α_2 -adrenergic agonist clonidine were measured in 25 depressed patients and 25 normal control subjects. Clonidine, by stimulating central inhibitory α_2 -adrenergic receptors, decreases central noradrenergic firing and turnover. The central effects of clonidine, in turn, lead to a decrease in sympathetic outflow. In the control subjects clonidine reduced plasma norepinephrine, blood pressure, and heart rate significantly more than found with placebo. In the depressed patients, clonidine reduced blood pressure and the percent fall in plasma norepinephrine, but not plasma MHPG or heart rate, significantly more than placebo. The absolute and percent reductions in plasma MHPG and heart rate following clonidine were significantly less in the depressed patients than in control subjects. These results raise the possibility that the sensitivity of the α_2 -adrenergic receptors inhibitory to noradrenergic output may be reduced in depression. Although the decreased responses to clonidine were significant for the entire group of depressed patients, they seemed to be largely attributable to a marked difference in the unipolar depressed patient subgroup. We have previously suggested that a "high output-low sensitivity" depression (i.e., increased noradrenergic output associated with reduced receptor responsiveness) may be observed primarily in unipolar patients, while bipolar patients may show low output and, in some cases, increased noradrenergic receptor responsiveness, and the current data seem to be at least partially consistent with this hypothesis.

Two intensively studied patients provided more detailed support for the hypothesis of a high catecholamine output state accompanied by reduced receptor sensitivity in unipolar depressed patients, supporting our earlier larger sample studies based upon plasma catecholamine and urinary MHPG measurements. These two unipolar postmenopausal depressed patients had extreme elevations in plasma norepinephrine concentrations (4-5 times higher than means for controls or a mixed sample of depressed patients) accompanied by reduced growth hormone responses to clonidine and reduced blood responses to tyramine. These latter two findings are consistent with α -adrenergic receptor subsensitivity.

When plasma cortisol responses to the intravenous administration of clonidine hydrochloride and placebo were evaluated in depressed patients and controls, the depressed patients had higher mean baseline cortisol levels than controls. Cortisol levels decreased during the morning study period following both placebo and 2 $\mu\text{g}/\text{kg}$ of clonidine hydrochloride in the depressed patients, but the cortisol decrease was sixfold greater on the day of clonidine administration; these placebo-clonidine differences were statistically significant, whether calculated on an absolute decrement basis or as a percent change. In contrast, controls responded to clonidine with only a 1.5-fold greater cortisol reduction than that found after placebo, a nonsignificant difference from the day of placebo administration. Reductions in the concentration of plasma 3-methoxy-4-hydroxyphenylglycol following clonidine administration were significantly negatively correlated with baseline plasma cortisol levels, raising the possibility that abnormalities in the responsiveness of the α_2 -noradrenergic system may be associated with the hypothalamo-pituitary-adrenal axis dysfunction found in depressed patients.

In studies of the mechanism of action of antidepressant drugs, the selective monoamine oxidase type A-inhibitory antidepressant, clorgyline, was found in electrophysiologic studies to slow the firing rate of locus ceruleus neurons in rat brain. This supports earlier data obtained with other antidepressants. This alteration precedes β - and α_2 -adrenergic receptor downregulation, and is thought to represent an intermediate stage in the adaptive events following MAO-inhibitor treatment. Although we and others have described brain receptor changes in rats, and cerebrospinal fluid amine metabolite changes in monkeys following clorgyline, no change in platelet α_2 -adrenoceptors or in the cyclic AMP response to norepinephrine was observed in depressed patients receiving clorgyline chronically. As we have also observed reduced blood pressure responses to clonidine in depressed patients receiving clorgyline, a finding suggestive of a functional α_2 -adrenergic down-regulatory response in man, the lack of change in platelet receptors and cyclic AMP responsiveness in the platelet studies suggests that this cell is not a reliable model for receptor studies of antidepressant drug effects in the brain.

Significance to Biomedical Research and the Program of the Institute:

The neuroendocrine, amine metabolite and blood pressure response differences to several drugs with highly selective actions on brain catecholamine and indoleamine systems provide additional evidence for alterations in the status of these important monoamine pathways in depressed patients. They go beyond static amine or metabolite measurements in providing evidence of disturbed function in these pathways. Our studies of antidepressant drug effects in animals indicate that these drugs affect the same α_2 -adrenergic response mechanisms which are altered in depressed patients.

Proposed Course:

Further work like that accomplished with adrenergic functions is needed to evaluate whether antidepressant drugs affect brain serotonin function in depressed patients. In addition, possible primary alterations in pituitary or hypothalamic neuroendocrine responsivity (rather than in the monoamine pathways) need to be evaluated in patients prior to and during antidepressant drug treatment.

Publications:

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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00329-09 CN

PERIOD COVERED October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Platelets and other systems as models for the study of neurotransmitter function

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Jonathan L. Costa, Staff Physician

CNB, NIMH

COOPERATING UNITS (if any)

LPD, NIAID; LCS, NIMH; CPB, NHLBI;
Hopital St. Louis, Paris

USUHS; Lab. de Biochimie,

LAB/BRANCH

Clinical Neuropharmacology Branch

SECTION

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

2.0

PROFESSIONAL:

1.5

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Techniques for studying the ultrastructure of resting platelets and activated platelets have been applied to cells after varying types of platelet storage. Isolated microvessels appear to retain some degree of autonomic innervation, which may be responsible for their ability to take up and store serotonin in a reserpine-sensitive compartment. Phenolsulfotransferase in human platelets, when assayed with the use of membrane-lysis agents, appears to act on some prostaglandin precursors and may alter the platelet response to stimulation. Tricyclic anti-depressants and their metabolites appear to inhibit a proton-pumping ATPase of Tower eukaryotes and to uncouple mitochondrial oxidative phosphorylation in human platelets.

Other collaborative professional personnel engaged on the project:

E. C. Weinbach	Section Chief	LPD, NIAID
D. J. Jacobowitz	Section Chief	LCS, NIMH
A. Robinson	Guest Worker	CPB, NHLBI
D. Zilberstein	Staff Fellow	LPD, NIAID
W. Scheibel	Associate Professor	USUHS
J.-M. Launay	Asst. des Hopitaux	Lab. de Biochimie Hopital St. Louis Paris, France

Project Description:

Objectives: To explore the functional parameters of human platelets, with special reference to the roles of energy metabolism and the enzyme phenolsulfotransferase in amine uptake and storage. In addition, the mechanisms of action of various psychoactive compounds are examined by studying the effects of these drugs on energy metabolism in mammalian mitochondria, pathogenic protozoa, and platelets.

Studies Implemented: (1) Use of platelet whole mounts and electron microscopy to evaluate platelet morphology and function after storage under varying conditions; (2) light-microscopic localization of serotonin accumulated by isolated microvessels (capillaries); (3) effects of psychoactive drugs and compounds which interfere with sterol metabolism on the viability and growth of pathogenic eukaryotes (Leishmania, Giardia, Plasmodium); (4) evaluation of the potential of leukotrienes, prostacyclins, and prostaglandins to serve as substrates for platelet phenolsulfotransferase; (5) characterization of the mitochondrial and extra-mitochondrial sources of energy in human platelets, and evaluation of the effects of psychoactive drugs on these parameters.

Methods Employed:

General Preparative Procedures. Platelets were isolated from normal volunteers by differential centrifugation or by plateletpheresis. Mitochondria were prepared by homogenization and differential centrifugation. Free-living eukaryotes were grown in axenic cultures, and Leishmania either in pure cultures or in mouse peritoneal macrophages. Plasmodium was grown in a culture medium containing human erythrocytes. Microvessels were isolated by homogenization and centrifugation over Percoll gradients.

Platelet Storage: Platelet concentrates in plasma were stored in different types of containers for varying periods of time. Aliquots were removed and platelet whole mounts made utilizing electron-microscope grids. Platelets in some aliquots were allowed to air dry, or to attach and spread on the carbon film prior to preparation of whole mounts. Whole mounts were examined and photographed in an electron microscope.

Amine Localization in Microvessels: Isolated microvessels were incubated with serotonin, and whole mounts were prepared on glass slides. After exposure to formaldehyde vapor, preparations were studied and photographed in a fluorescence microscope.

Drug Effects on Pathogenic Eukaryotes: Energy metabolism in eukaryotic cells was assessed by measuring oxygen and proton uptake in a polarographic instrument, and ATPase activity by biochemical methods.

Non-Amine Phenolsulfotransferase Substrates: The permeability of the platelet membrane was increased by exposure to a hypotonic medium, by sonication, by addition of nystatin and toluene, and by incubation with

bacterial sulfhydryl-activated toxins. Phenolsulfotransferase activity was measured utilizing sulfur-35 labelled PAPS as a sulfate donor and a variety of prostaglandins, leukotrienes, and metabolites as substrates.

Energy Metabolism in Human Platelets: Oxygen consumption and proton production were examined utilizing a polarographic instrument. Platelets were induced to depend on mitochondrial energy production by addition of 2-deoxyglucose and pyruvate.

Major Findings:

Platelet Storage: Platelets stored in gas-permeable plastic bags maintain a higher pH and oxygen tension than those stored in conventional bags. After 3 days of storage, however, the cells in permeable bags exhibit a variety of morphologic abnormalities which may be induced by the presence of residual plasticizer in the bags. The effects of these morphologic changes on platelet spreading, activation, and dense-body release are currently being evaluated.

Amine Localization in Microvessels: Microvessels isolated from the rat fat pad are associated with small fragments of adrenergic nerves. Varicosities on these fibers take up serotonin when the preparation is incubated with exogenous serotonin, and may be responsible for the reserpine-sensitive uptake and storage described previously.

Drug Effects on Pathogenic Eukaryotes: Both Giardia and Leishmania organisms are inhibited by chlorimipramine and by certain of its analogues and metabolites, although both cyanimipramine and imipramine are much less effective on a molar basis. Chlorimipramine appears to interfere with a membrane-associated ATPase responsible for pumping protons. Giardia grows best in the presence of exogenous cholesterol and vitamin E, and is not particularly sensitive to drugs which inhibit squalene epoxidation or lanosterol synthesis. Leishmania, in contrast, appears to require endogenous sterol synthesis for its growth and viability.

Non-Amine Phenolsulfotransferase Substrates: Phenolsulfotransferase activity with biogenic amine substrates may be assayed in platelets following sonication or resuspension in a hypotonic medium. These procedures result in essentially no activity when prostaglandins, prostacyclins, or leukotrienes are employed as substrates. Use of bacterial toxins to lyse platelet membranes produces an increase in measured enzyme activity. The best substrates for the enzyme appear to be prostaglandin precursor substances such as hydroxy eicosanoic acid derivatives (i.e., HETE).

Energy Metabolism in Human Platelets: The contribution of mitochondrial ATP production to platelet energy metabolism can be evaluated in intact platelets incubated with 2-deoxyglucose and pyruvate. The thrombin-induced stimulation of ATP production appears to increase in association with increased mitochondrial oxygen consumption. Chlorimipramine added in vitro also causes increased mitochondrial oxygen

consumption which does not return to baseline values after oligomycin addition, and appears to uncouple platelet respiration. Imipramine is less potent on a molar basis in producing the same effect.

Significance to Biomedical Research and the Program of the Institute:

Although platelet phenolsulfotransferase can sulfate intra-platelet dopamine and serotonin, its physiologic role in the disposition of these amines remains to be determined. If the enzyme can act to sulfate prostaglandin precursors as well, it may inactivate some of these compounds, and as a consequence could play an important role in regulation of secretion and aggregation. The Annual Reports for 1983 and this year have discussed data indicating that the tricyclic antidepressants can alter energy metabolism in both eukaryotic and mammalian systems. In order to explore the possible relevance of these observations in human systems adapted for amine storage and release, studies of effects of the compounds on the energy metabolism of isolated human platelets have been initiated. Once the system has been characterized in more detail, platelets obtained from patients receiving psychoactive medications can be studied as well.

Proposed Course:

Continue exploration of the physiologic role of phenolsulfotransferase in human platelets, since definition of its function in these cells will provide hypotheses about its possible significance in the brain. In addition, continued definition of platelet energy metabolism will provide a model with which to examine the energy-related effects of psychoactive compounds.

Publications:

Kwon-Chung, K.J., Tom, W.K. and Costa, J.L.: Utilization of indole compounds by Cryptococcus neoformans to produce a melanin-like pigment. J. Clin. Microbiol. 18: 1419-1421, 1983.

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Feder, R., Mayne-Banton, V., Sayre, D., Costa, J., Kim, B.K., and Baldini, M.G.: Recent developments in contact x-ray microscopy. In Schmalal, G. (ed.): Proceedings of Springer Series in Optical Sciences.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00330-06 CN

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Use of electron and photon imaging techniques to study aminergic systems

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Jonathan L. Costa, Staff Physician, CNB, NIMH

COOPERATING UNITS (if any)

Harvard Medical School, New England Nuclear Corp., IBM Research Center, SUNY at Stonybrook, Brookhaven National Laboratory, Los Alamos National Laboratory, Maxwell Laboratories

LAB/BRANCH

Clinical Neuropharmacology Branch

SECTION

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

2.0

PROFESSIONAL:

1.5

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The groundwork has been laid for x-ray holography of biological specimens by producing holograms of small specimens and by optimizing the arrangement of source and specimen. Contact x-ray microscopy of air-dried platelets and platelet subcellular components continues to document unusual patterns of photon absorbance which do not correlate well with electron scattering power. In particular, thrombin-stimulated platelets have a small, very dense core from which all pseudopods radiate. Hydrated (living) platelets have also been imaged utilizing flash x-ray microscopy, and appear to possess several features, (e.g. pseudopod-associated granules) not seen in air-dried cells. Synchrotron radiation tuned monochromatically has been used to examine the elemental composition of platelets and bacteria. In Corynebacterium, calcium-rich structures lie adjacent to the bacterial cytoskeleton, and in platelets the cytoskeleton appears to be rich in oxygen and phosphorous.

Other collaborative professional personnel engaged on the project:

M. Baldini	Professor	Harvard Medical School
R. Feder	Staff Scientist	IBM Research Center
J. Kirz	Professor	SUNY, Stonybrook; Brookhaven
S. Layne	Visiting Associate	Los Alamos National Laboratory
J. Pearlman	Scientist	Maxwell Laboratories
D. Sayre	Staff Scientist	IBM Research Center
J. Solem	Staff Scientist	Los Alamos National Laboratory
J. Webster	Research Scientist	New England Nuclear Corp.

Project Description:

Objectives: To assist in the development of techniques which permit imaging of living or dehydrated biological specimens with ultrastructural resolution, and to couple this capability to the delineation of the elements present in various subcellular areas. In addition, once the methods are reproducible, to apply them to the study of human platelets and other amine-storing systems.

Studies Implemented: (1) Exploration of the utility of various types of x-ray sources for x-ray holography of biological specimens; (2) development of the instrumentation necessary to carry out holography utilizing an x-ray laser; (3) continuing studies of hydrated cells with gas-jet pulsed plasma x-ray sources; (4) elemental localization in biological specimens utilizing Synchrotron radiation; (5) contact x-ray microscopy of human platelet preparations.

Methods Employed:

1. General Preparative Procedures: Whole blood was collected from normal volunteers or from patients with sickle-cell disease. Platelets and red blood cells were prepared by differential centrifugation. Bacteria were grown on agar plates or in broth cultures.

2. Source Development for X-Ray Holography: Bacteria, red blood cells, and platelets were prepared as air-dried whole mounts or were maintained in the hydrated state. Specimens were placed in contact with x-ray-sensitive resists and exposed to intense pulses of x-rays generated during the interaction of light from carbon dioxide lasers with a small target. Various specimen holder configurations were also tested for their ability to maintain cells in a wet state and to permit holographic recording. Sample x-ray holograms and x-ray diffraction patterns were generated by allowing Synchrotron radiation to interact with specimens of known geometry and composition.

3. Pulsed-Plasma X-ray Microscopy: Dried and hydrated specimens (bacteria, red blood cells, and platelets in the unstimulated state and after various types of activation) were placed in contact with resist inside a specially fabricated chamber. They were then exposed to intense pulses of long wavelength x-rays, generated in a gas-jet, pulsed-plasma x-ray source.

4. Elemental Localization with Synchrotron Radiation: Air-dried whole mounts of bacteria and platelets were placed in contact with a series of resists, which were exposed utilizing x-ray wavelengths just above and below the K- and L-absorption edges of various elements (e.g. calcium, carbon, nitrogen, and oxygen). Platelets loaded with fluorinated biogenic amines were replicated with x-rays above and below the fluorine K edge.

5. Contact Microscopy of Platelet Preparations: Various types of platelet preparations were exposed to carbon K-edge x-rays generated in an electron-gun source. Samples examined included platelet microtubule bundles, platelet actin rings, and whole mounts and thin sections of platelets in varying stages of activation.

Major Findings:

1. Source Development for X-Ray Holography. Holographic patterns can be generated when Synchrotron radiation interacts with micron-sized specimens. Utilization of this technique will permit testing of various physical arrangements for obtaining the hologram, as well as the development of computer programs appropriate for reconstruction. A unique type of holographic setup appears to work best with an x-ray source similar in many ways to an x-ray laser--namely the exposure of a small target to a focused array of beams produced by a series of carbon dioxide lasers. This system produces acceptable images of red blood cells and bacteria, although the concomitant production of heavy ions appears to degrade the image quality by partially exposing the resist.

2. Hydrated Platelets Studied by Flash X-Ray Microscopy. Living, unstimulated (resting) platelets have an x-ray ultrastructure which corresponds closely with that seen in whole mounts of air-dried platelets. Pseudopods on mildly stimulated platelets contain a central core of photon-absorbent material which extends into the platelet cytoplasm and appears to connect with a cytoskeletal network. Small flocculent granules, not previously visualized in air-dried specimens, are associated in some cases with pseudopod bases or lie close to granular structures. When platelets are stimulated maximally with high doses of thrombin, a series of pseudopods radiate out from a very dense central core.

3. Elemental Composition of Bacteria and Platelets. The "cytoskeletal" core inside Corynebacterium absorbs photons strongly on both sides of the carbon and oxygen K-edge positions, suggesting that it is rich in carbon, oxygen, nitrogen, and phosphorus. When imaged with x-rays just above the calcium L-edge, the bacteria contain small structures which are at right angles to the central core and lie immediately under the cell wall plasma membrane area. Since these areas are not visible when bacteria are imaged with x-rays below the calcium edge, they appear to contain high concentrations of calcium. The platelet cytoskeletal network, in contrast, presents a different type of arrangement depending on which wavelength is used for replication. Studies of the physiologic significance of these structural differences are underway.

4. Contact Microscopy of Human Platelets. Isolated bundles of platelet microtubules and actin filaments replicate with carbon K-edge radiation as unique morphological entities, with an x-ray absorbant structure which is at variance with their patterns of electron opacity. Platelets stimulated with thrombin centralize most of their electron-opaque substance and send out multiple pseudopods; a small central core absorbs carbon x-rays very strongly.

Significance to Biomedical Research and the Program of the Institute:

Although x-ray microscopy and spectroscopy are technically difficult, both techniques offer significant advantages over similar methods utilizing electrons. The most well-understood and simplest of the x-ray techniques, contact x-ray microscopy with an electron-gun source, has been used to correlate structure and formation in resting platelets, stimulated

platelets, and platelet subcellular components. Study of similar specimens with Synchrotron radiation, which permits assessment of the elemental composition of various x-ray opaque structures, can be expected to provide further insight into their putative functions. Extrapolation to hydrated (living) platelets with a flash x-ray source is also technically feasible. X-ray holography of living specimens, utilizing an intense x-ray laser as a source, is the most attractive because it appears to combine the advantages of the other x-ray techniques. It will not be possible to realize those benefits, however, without model studies such as those described here, which explore the optimal holographic setup, hologram processing techniques, and the design of appropriate holders for biological specimens.

Proposed Course:

Continue utilizing a combination of x-ray techniques to understand the morphological changes associated with amine uptake and storage in human platelets. Of particular interest are studies on the subcellular localization of ring-fluorinated biogenic amines.

Publications:

Baldini, M.G., Kim, B.K., Feder, R., Sayre, D., Mayne-Banton, V., and Costa, J.L.: Possibilities for the study of blood platelets using soft x-ray microscopy. In Himpsel, F.J., and Klaffky, R.W. (Eds.): Proceedings for the International Society for Optical Engineering, 447: 164-171, 1983.

Feder, R., Mayne-Banton, V., Sayre, D., Costa, J., Kim, B.K., Baldini, M.G., and Cheng, P.C.: Recent developments in x-ray contact microscopy. In Schmahl, G., and Rudolph, D. (Eds.): X-Ray Microscopy. Proceedings of the International Symposium. Gottingen, Federal Republic of Germany, 1983, pp. 279-284.

Webster, J.A., Fay, D.D., Costa, J.L., Jones, P.M., and Hugh, R.: Elemental composition of bacterial metachromatic inclusions determined by electron microprobe x-ray analysis. J. Bacteriol., 158: 441-446, 1984.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00331-06 CN

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Use of nuclear magnetic resonance to study aminergic systems

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Jonathan L. Costa, Staff Physician, CNB, NIMH

COOPERATING UNITS (if any)

LC, NHLBI; LC, NIAODK; LCS, NIAAA

LAB/BRANCH

Clinical Neuropharmacology Branch

SECTION

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

2.0

PROFESSIONAL:

1.5

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The technique of nuclear magnetic resonance has been used to examine the properties of fluorinated amines inside platelets. In pig platelets, fluorinated quinacrine added to the vesicles assumes motional properties similar to those of the other vesicular contents. In human platelets, 5-fluorodopamine sulfate appears to be retained in the cytoplasm. Proton nuclear magnetic resonance studies of amino acids and glucose metabolism in cells and living tissues appear to be feasible. Alcohol alters the relaxation behavior of water protons in red blood cells and plasma.

Other collaborative professional personnel engaged on the project:

K. L. Kirk	Chemist	LC, NIADDK
E. A. Sokoloski	Chemist	LC, NHLBI
M. Linnoila	Lab Chief	LCS, NIAAA

Project Description:

Objectives: (1) To continue studies of the storage mechanisms for amines in pig and human platelets by observing fluorinated compounds with nuclear magnetic resonance; (2) to explore the use of nuclear magnetic resonance as a method of studying the behavior of water protons and other protons in living systems.

Studies Implemented: (1) Characterization of the molecular mobility of difluoroserotonin and ring-fluorinated quinoline (fluoquine) in storage vesicles of pig platelets; (2) examination of the motional characteristics and chemical state of 5-fluorodopamine inside human platelets; (3) study of the relaxation behavior of water protons in blood following ethanol addition or ingestion; (4) exploration of the use of a high-field spectrometer for study of water and other protons in living systems.

Methods Employed:

1. General Preparative Procedures. Whole blood was collected from humans and pigs into commercially-available bags, or into small tubes containing anticoagulant. In some cases, platelet-rich plasma was collected by plateletpheresis. Red blood cells, platelet-rich plasma, and platelet-poor plasma were prepared by differential centrifugation.

2. NMR Examination of Platelets. Platelets were resuspended in buffer at a density of 10^{10} cells per ml and studied utilizing a ^{19}F probe. In some aliquots, temperature was varied or thrombin and the ionophore X537A were added sequentially.

3. NMR Examination of Protons. Protons were studied at both high and low fields utilizing a ^1H probe. In some cases, ethanol or varying amounts of D_2O were added to solutions prior to examination. The spin-lattice relaxation times (T_1) were estimated utilizing an inversion-recovery pulse sequence, and varying types of pre-saturation pulses were employed in order to suppress the signal from water protons.

Major Findings:

1. Molecular Mobility of Amines in Pig Platelets. Previous work in this laboratory has shown that the core material in pig platelet storage vesicles exists in a gel-like state at 4°C , and that the gel progressively dissolves (becomes less viscous) as the temperature is raised to 37°C . This can be monitored by changes in the linewidths of both intra-vesicular nucleotides and analogues of biogenic amines (fluorinated serotonin and dopamine). Addition of quinacrine to the vesicles leads to an increase in the relative molecular mobility of all other vesicular components between 4°C and 37°C . The current work documents the fact that both the fluorinated quinacrine and serotonin in pig platelets have the same linewidth temperature dependence, and that both are relatively more mobile at all temperatures than is fluorinated serotonin in the absence of quinacrine. The addition of quinacrine to the vesicles thus appears to participate directly in and become a part of the gel structure, rather than perturbing its physical characteristics in a non-specific fashion.

2. Molecular Characteristics of 5-Fluorodopamine in Human Platelets.

Approximately 50% of the dopamine taken up by human platelets resides in an extra-vesicular (cytoplasmic) compartment. When platelets are loaded with 5-fluorodopamine and observed by ^{19}F -NMR, a fluorine peak with a chemical shift distinct from that of the parent compound is visible. The peak is not lost from the cells following release of vesicular amine with either thrombin or the ionophore X537A, and has a narrow linewidth at 4°C . If the peak is associated with 5-fluorodopamine sulfate, our NMR observations suggest that this compound resides relatively free (i.e. unbound) in the platelet cytoplasm. Vesicular 5-fluorodopamine appears to be essentially immobilized, as are ring-fluorinated serotonins.

3. Relaxation of Blood Protons in the Presence of Ethanol.

Addition of ethanol to plasma at concentrations in excess of 0.5% increases the T1 (spin-lattice relaxation time) of the water. The effect is even more pronounced in buffer solutions, possibly because of their low protein content. In subjects who have ingested ethanol, the red-blood-cell T1 appears to decrease because of a slight degree of dehydration of the cells, while the plasma T1 increases to a small extent.

4. Proton NMR with a High-Field Spectrometer.

By utilizing an appropriate sequence of pre-saturation and pulse-suppression sequences, it is possible to obtain signals from non-water protons in solutions containing up to 90% water. It should therefore be possible to examine the protons in various types of cells, as well as those in the brains and other organs of intact animals. In addition, the T1's of body fluids and tissues may be examined utilizing the proper techniques.

Significance to Biomedical Research and the Program of the Institute:

Other workers have reported that high-field NMR may be used to study amino acid sequestration, glucose metabolism, and lactic acid production in the brains of living animals. The present work shows that similar studies are possible with the spectrometer available to the NIMH. Since ethanol ingestion by normal volunteers apparently produces changes in the relaxation behavior of water in both red blood cells and plasma, further study of blood and tissues from normals and alcohol addicts may be indicated. NMR imaging of the brain in these types of humans may be particularly interesting. NMR studies of platelets loaded with fluorinated amines document the potential of this technique to examine unique types of storage mechanisms for different types of amines. Fluorinated quinolines, for example, appear to alter the nature of the entire amine storage complex in vesicles of pig platelets, and 5-fluorodopamine O-sulfate may be retained in human platelets primarily because the anion is unable to diffuse or be transported across the plasma membrane. Application of similar techniques should enhance understanding of the processes of amine retention by other aminergic tissues.

Proposed Course:

Utilize ring-fluorinated quinoline, dopamine, norepinephrine, and serotonin to examine uptake and storage by nerve microsacs. Examine amino

acid and glucose metabolism in isolated platelets by proton NMR; once the techniques have been worked out in detail, apply these to the study of similar functions in other amine-storing cells and tissues or to the brain in intact animals. Continue exploration of the effects of ethanol on blood cells and tissues, with the anticipation of extending the studies with nuclear magnetic resonance imaging.

Publications:

Costa, J.L., Fay, D.D. and Kirk, K.L.: Quinacrine and other basic amines in human platelets: Subcellular compartmentation and effects of serotonin. Res. Commun. Chem. Pathol. Pharmacol. 43: 25-42, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00332-06 CN

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Animal Models for the Study of Neuropharmacologic Effects

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Charanjit S. Aulakh Visiting Associate, CNB, NIMH

COOPERATING UNITS (if any)

LPP, NIMH; Howard University

LAB/BRANCH

Clinical Neuropharmacology Branch

SECTION

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

1.3

PROFESSIONAL:

0.8

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The time-dependent effects of the selective monoamine oxidase (MAO) type A-inhibiting antidepressant clorgyline were studied in rodents. Functional adaptive changes in both the noradrenergic and serotonergic neurotransmitter systems in the central nervous system were observed to develop following long-term administration of clorgyline. The importance of these adaptive changes in understanding the molecular mechanisms responsible for the efficacy of antidepressants is supported by our recent finding that self-stimulation behavior in rodents was enhanced only after long-term administration of clorgyline. The slow return of adaptational changes following discontinuation of clorgyline observed recently has implications for understanding some delayed drug interactions associated with MAO-inhibiting antidepressants in man.

Other collaborative professional personnel engaged on the project:

D. L. Murphy	Chief	CNB NIMH	.
R. M. Cohen	Section Chief	LPP NIMH	
S. N. Pradhan	Professor	Howard University	

Objectives

The therapeutic effects of clinically effective antidepressant drugs are not observed until two or more weeks of their administration, whereas inhibition of uptake or enzymatic inhibition occurs immediately after the first injection. Therefore, recent pharmacological studies have focused on the adaptive changes in the central nervous system following long-term administration of antidepressant drugs. These adaptive changes might be important in understanding the molecular mechanisms responsible both for the efficacy and the side effects of these drugs. With this approach in mind, we have conducted a series of studies with the monoamine oxidase type A-inhibiting antidepressant clorgyline in the rodents with the expectation that these studies will enhance our understanding of etiology and treatment of affective disorders.

Methods Employed:

For studies of self-stimulation behavior, bipolar stainless steel electrodes are implanted stereotaxically in different brain regions. Following surgery, the animals are trained to press a lever in a Skinner box in order to receive reinforcement from intracranial electrical stimulation.

Locomotor activity is measured with Animex activity meters. For food intake studies, the animals are trained to take their daily food from 09:00 to 13:00 h. At the end of the first hour of food access, the remaining food is weighed and the difference from the original amount constitutes the measure of food intake.

Receptors from crude brain homogenates are measured by standard radioactive ligand assays. [^3H]-WB 4101, [^3H]-clonidine and [^3H]-dihydroalprenolol are the specific ligands used for the measurement of α_1 -, α_2 - and β -adrenoreceptors respectively.

Major Findings:

Treatment for 21 days but not 3 days with clorgyline (1 mg/kg/day), a selective monoamine oxidase type A inhibitor with antidepressant effects, significantly attenuated m-chlorophenylpiperazine's (m-CPP) effects on food intake, sedation level and induction of limb movements, but sensitized rats to ejaculation. m-CPP is a serotonergic receptor agonist. These findings demonstrate functional serotonin pathway adaptational changes in response to antidepressant drug treatment. Long-term treatment with clorgyline also facilitated hypothalamic self-stimulation behavior in rats, while acute clorgyline treatment was without effect. Furthermore, long-term but not short-term clorgyline treatment significantly attenuated the suppressive effect of the selective α_2 -adrenergic agonist clonidine on this behavior.

Also, at 21 days, but not at 7 days, clorgyline caused significant escape from clonidine's normal suppressant effect on locomotion in the rat,

along with decreases in the number of α_2 -adrenoreceptors in the cortex; these changes revert towards pretreatment values very gradually over an eight week period following discontinuation of the drug.

Significance to Biomedical Research and the Programs of the Institute:

The functional adaptational changes in both the noradrenergic and serotonergic pathways following long-term treatment with an antidepressant drug observed in the present report are important since both of these neurotransmitter systems have been implicated in the etiology of the affective illnesses. Which one of these two mechanisms is the final mediator of antidepressant effects is a question of further investigation. The present data demonstrating the slow return of adaptational changes following discontinuation of an antidepressant drug in animals have implications for understanding some delayed drug interactions associated with MAO-inhibiting antidepressants in man.

Proposed Course:

During the next year, we will be attempting to explore more of the functional adaptational changes in the serotonergic system using other classes of antidepressant drugs as well as other animal behavioral models. We will also want to examine the interrelationships between the serotonergic and noradrenergic systems in adaptational processes accompanying antidepressant administration.

Publications:

Aulakh, C.S., Cohen, R.M., Pradhan, S.N. and Murphy, D.L.: Self-stimulation responses are altered following long-term but not short-term treatment with clorgyline. Brain Res., 270:383-385, 1983.

Aulakh, C.S., Cohen, R.M., McLellan, C. and Murphy, D.L.: Correlation of changes in α_2 -adrenoceptor number and locomotor responses to clonidine following clorgyline discontinuation. Br. J. Pharmacol., 80:10-12, 1983.

Cohen, R.M., Aulakh, C.S. and Murphy, D.L.: Long-term clorgyline treatment antagonizes the eating and motor function responses to m-chlorophenyl-piperazine. Eur. J. Pharmacol., 94:175-179, 1983.

Cohen, R.M., and Campbell, I.C.: Receptor adaptation in animal models: A state change approach to psychiatric illness. In Post, R.M., and Ballenger, J.C. (Eds.): Neurobiology of the Mood Disorders, Baltimore, Williams and Wilkins Co., pp. 572-586, 1984.

Murphy, D.L., Garrick, N.A., Aulakh, C.S. and Cohen, R.M.: New contributions from basic science to understanding the effects of monoamine oxidase inhibiting antidepressants. J. Clin. Psychiatry, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00336-05 CN

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The phenomenology and treatment of obsessive-compulsive disorder in adults

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Thomas R. Insel, Staff Physician, Clinical Neuropharmacology Branch, NIMH

COOPERATING UNITS (if any)

LPP, LCS, NSB, NIAA

LAB/BRANCH

Clinical Neuropharmacology Branch

SECTION

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS

3.2

PROFESSIONAL:

1.8

OTHER:

1.4

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Summary

Obsessive-compulsive disorder has been studied from several different perspectives since the beginning of this project in 1980. During the past year, the major focus has been to test the hypothesis that serotonergic function might be altered in patients with this disorder. This hypothesis, which was generated from several promising clinical trials with the tricyclic antidepressant clomipramine, was examined by (1) measuring the serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA) in cerebrospinal fluid (2) assaying serotonin uptake and [³H]-imipramine binding in platelets, and (3) comparing neuroendocrine response to the serotonergic agent fenfluramine in obsessional patients and matched controls. On each of these measures, the patients were not significantly different from controls. Furthermore, in a double blind treatment comparison of the serotonin uptake inhibitor zimelidine and the noradrenergic uptake inhibitor desipramine, neither drug was found to be as effective as clomipramine. These findings suggest that serotonin uptake, turnover, and receptor sensitivity are not altered in obsessional patients, and that pharmacologic blockade of serotonin uptake is not sufficient for the reduction of obsessional symptoms.

Other collaborative professional personnel engaged on the project:

E. A. Mueller	Staff Physician	CN NIMH
D. L. Murphy	Chief	CN NIMH
J. L. Rapoport	Section Chief	LCS NIMH
M. Linnoila	Section Chief	LCS, NIAAA
T. Zahn	Staff Psychologist	LPP NIMH
S. M. Paul	Chief	CNB NIMH

Project Description:

Objectives. Over the past four years we have developed a comprehensive research program to investigate both psychological and biological aspects of obsessive-compulsive disorder. Our initial objectives focused on descriptive or diagnostic aspects of the syndrome: Is the disorder homogeneous or are there a variety of syndromes within this disorder with heterogeneous responses to treatment? In the second and third year of research our investigations focused more on the treatment of the disorder: Do patients with this disorder respond to antidepressants and if so, what biologic markers might predict drug response? Our results suggested that the disorder was heterogeneous and that clomipramine, a tricyclic antidepressant with potent inhibitory effects on the neuronal re-uptake of serotonin would reduce obsessional symptoms in a wide spectrum of obsessional patients. In the past year we have begun to study serotonergic function in obsessional patients. Our specific hypothesis was that obsessionals would manifest alterations in serotonin uptake or turnover similar to previous findings in other disorders involving deficits in the control of affect, impulse, and aggression. A related objective was to determine if an antidepressant drug that selectively blocked neuronal re-uptake of serotonin would be more effective clinically than an antidepressant that selectively blocked re-uptake of norepinephrine.

Methods Employed:

This study is being conducted in the NIMH outpatient clinic at the Clinical Center. Local patients with obsessive-compulsive disorder are accepted if they have been ill for at least one year and are willing to stop all psychotropic medications. In place of the extensive "baseline" testing completed in previous years, patients are studied on parameters directly relevant to neurotransmitter function. These studies include: (1) [³H]-imipramine binding and serotonin uptake in platelets, (2) sampling of lumbar cerebrospinal fluid for brain amines and their metabolites, and (3) measuring neuroendocrine response to the serotonergic agent fenfluramine. Platelet and cerebrospinal fluid studies are repeated after five weeks of treatment on one of two selective antidepressants. Each patient is randomly assigned to double-blind treatment with either zimelidine (a selective serotonin re-uptake inhibitor) or desipramine (a selective norepinephrine re-uptake inhibitor). Following a five-week trial on one of these drugs, patients cross over to a five-week trial with clomipramine.

Major Findings:

Forty patients with obsessive-compulsive disorder have been studied over the past four years. The first generation of studies which ended in 1982 documented the effectiveness of clomipramine for patients with this disorder. Twelve patients studied in the double-blind crossover comparison of clomipramine and clorgyline revealed consistent improvement on measures of obsessions, depression, and anxiety after four and six weeks of clomipramine treatment but no significant changes after equal periods of treatment with clorgyline (n = 11) or following four weeks of placebo administration (n = 13). Improvement with clomipramine treatment was not limited to

patients with secondary depression, nor was it predicted by the type of obsessions (e.g., washing vs. checking). In addition, there was a significant correlation between improvement in obsessional symptoms and plasma levels of clomipramine. Follow-up of patients continued on clomipramine after six months and one year of treatment shows persistent and progressive improvement.

A recent detailed analysis of the response to clomipramine and clorgyline has been possible with psychophysiological tests completed across the stages of the crossover study. Serial studies of galvanic skin response reveal that both clomipramine and clorgyline administration are associated with lower levels of arousal but only clomipramine decreases the physiologic response to aversive stimuli, such as a loud noise. This change in psychophysiological responsiveness is significantly correlated with improvement in obsessional symptoms and thus may reflect an important aspect of clomipramine's therapeutic effect in obsessive-compulsive disorder.

In the past year fourteen additional patients have entered the project. Results from our recent studies show that, contrary to the serotonin hypothesis, obsessional patients do not differ from controls on measures of platelet [³H]-imipramine binding or serotonin uptake (n = 12), nor do they show lower levels of the CSF serotonin metabolite 5-hydroxy-indoleacetic acid (5-HIAA) (n = 9). The plasma prolactin response to fenfluramine, previously hypothesized to be a serotonergic effect, does not appear to be significantly blunted in these obsessional patients (n = 8). These results, with a small group of obsessional outpatients, fail to demonstrate an alteration in serotonin uptake or turnover. In addition, the fenfluramine prolactin response suggests that post-synaptic serotonin receptor sensitivity is not reduced in obsessional patients. In addition, desipramine has not been effective as an anti-obsessional agent (n = 9), in spite of its well recognized antidepressant effects in affectively ill patients. Five patients received zimelidine prior to the drug being recalled by the manufacturer. Results from these five patients resembled the findings with the desipramine group. There was no significant decrease in obsessional symptoms, although even in this small sample clomipramine treatment led to significant improvement. These preliminary results do not support the hypothesis that serotonin uptake is abnormal in obsessional disorder or that the pharmacologic blockade of serotonin uptake is sufficient for an anti-obsessional effect. In contrast, to the treatment of affective illness, it appears that only certain tricyclic drugs have therapeutic effects in obsessional disorder.

Significance to Biomedical Research and the Program of the Institute:

These studies of obsessive-compulsive disorder continue a program of research into an important and poorly understood psychiatric syndrome. The importance of this syndrome resides not only in its debilitating and treatment refractory nature, but in its unique intermingling of affective and cognitive symptoms and in its highlighting intrapsychic conflicts over

aggressive impulses. By establishing a focus of interest in this disorder, the NIMH has become one of two major referral centers for obsessional patients from across the country.

Proposed Course:

The mechanism of clomipramine's anti-obsessional effect remains to be elucidated. As this drug appears to be selective among antidepressants for reducing obsessions, one strategy will be to determine, in the laboratory, what further characteristics other than serotonin uptake inhibition might distinguish this drug from structurally similar compounds. Another focus in the coming year will be to look more closely at correlates of clinical response with clomipramine. In particular, we plan to study cerebral blood flow to investigate regional cortical function in obsessionals before and after clomipramine treatment.

Publications:

Insel, T.R., Hamilton, J., Guttmacher, L., and Murphy, D.L.: D-amphetamine in obsessive-compulsive disorder. Psychopharmacology, 80: 231-235, 1983.

Insel, T.R., Donnelly, E.F., Lalakea, M.L., Alterman, I.S., and Murphy, D.L.: Neurological and neuropsychological studies of patients with obsessive-compulsive disorder. Biol. Psychiatry, 18: 741-751, 1983.

Insel, T.R., Hoover, C., and Murphy, D.L.: Parents of patients with obsessive compulsive disorder. Psychol. Med., 13: 807-811, 1983.

Siever, L.J., Insel, T.R., Jimerson, D.C., Lake, C.R., Uhde, T.W., Aloji, J. and Murphy, D.L.: Growth hormone response to clonidine in obsessive-compulsive patients. Br. J. Psychiatry, 142: 184-187, 1983.

Insel, T.R., Murphy, D.L., Cohen, R.M., Alterman, I., Linnoila, M. and Kilts, C.: Obsessive-compulsive disorder: A double-blind treatment trial of clomipramine and clorgyline. Arch. Gen. Psychiatry, 40: 605-712. 1983.

Insel, T.R. and Pickar, D.: Naloxone exacerbates obsessive doubt. Am. J. Psychiatry, 140: 1219-1220, 1983.

Insel, T.R. and Mueller, E.A.: Pharmacologic treatment of OCD. In Insel, T.R. (ed.): New Findings in Obsessive Compulsive Disorder, American Psychiatric Press, 71-88, 1984.

Insel, T.R.: Obsessive compulsive disorder: The clinical picture. In Insel, T.R. (ed.): New Findings in Obsessive Compulsive Disorder, American Psychiatric Press, 1-22, 1984.

Insel, T.R., Zahn, T. and Murphy, D.L.: Obsessive compulsive disorder: An anxiety disorder. In Mazer, J. and Tuma, H. (eds.): Anxiety and Anxiety Disorders, Lawrence Earlbaum Press, in press.

Insel, T.R., Mueller, E.A., Gillin, C., Siever, L.J. and Murphy, D.L.:
Biological markers in obsessive compulsive and affective disorders.
J. Psychiatric Res., in press.

Insel, T.R., Mueller, E.A., Gillin, C., Siever, L.J. and Murphy, D.L.:
Tricyclic response in obsessive compulsive disorder. Prog.
Neuropsychopharmacol. and Biol. Psychiat., in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00337-05 CN

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neuropharmacology of neuroendocrine and neurotransmitter regulatory mechanisms

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Dennis L. Murphy, M.D., Chief, Clinical Neuropharmacology Branch, NIMH

COOPERATING UNITS (if any)

Centre for Reprod. Biology, Edinburgh, Scotland; LN, LCS, NIMH; DBEB, NICHD

LAB/BRANCH

Clinical Neuropharmacology Branch

SECTION

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

0.8

PROFESSIONAL:

0.3

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In explorations of the influence of serotonin on neuroendocrine function, prolactin, growth hormone and cortisol concentrations were all found to be elevated in rhesus monkey plasma following intravenous administration of a selective serotonin receptor agonist, m-chlorophenylpiperazine, across a six-fold dosage range. These hormone changes were either completely blocked (in the case of prolactin and growth hormone) or partially inhibited (cortisol) by low concentrations of the serotonin antagonist, metergoline.

Other collaborative professional personnel engaged on the project:

J. A. Aloï	Chemist	CNB NIMH
N. A. Garrick	Biologist	CNB NIMH
T. R. Insel	Staff Physician	CNB NIMH
S. P. Markey	Unit Chief	LCS NIMH
M. Mishkin	Chief	LN NIMH
E. A. Mueller	Staff Fellow	CNB NIMH
L. Tamarkin	Staff Fellow	DBEB NICHD
P. Taylor	Chemist	Centre for Reprod. Biology, Edinburgh

Project Description:

Objectives: The discovery that a multitude of peripheral peptide hormones are present in high concentrations in the brain has led to an entire field of inquiry into the modulatory interactions between peptides, hormones and the classical monoamine neurotransmitters. This project has focused on the measurement of peptide hormones and monoamines and their metabolites in cerebrospinal fluid and plasma in an attempt to evaluate (a) diurnal changes; (b) define the relationship between plasma and CSF peptide levels; (c) evaluate their concentrations at different levels of CSF (e.g., lateral ventricular versus lumbar); and (d), in particular, to assess the effects of drugs which are known to affect monoamines using biochemical, behavioral and neuroendocrine response measures.

Methods Employed:

Cerebrospinal fluid from non-human primates is collected by means of indwelling lumbar or lateral ventricular cannulae for continuous flow into a refrigerated fraction collector. Monkey plasma is obtained either by use of indwelling venous catheters or by femoral venipuncture following ketamine-induced anesthesia. The following hormones are measured by radioimmunoassay: cortisol, prolactin, growth hormone, β -endorphin and melatonin (in collaboration with Larry Tamarkin, NICHD). Serotonin is measured by capillary mass spectrometry.

Major Findings:

In an attempt to develop possible serotonin system-selective agents for use in neuroendocrine challenge tests in man, we evaluated dose-response relationships of a putative serotonin postsynaptic receptor agonist, meta-chlorophenylpiperazine in rhesus monkeys. Changes in plasma prolactin, cortisol and growth hormone were measured in an initial set of studies in rhesus monkeys. Meta-chlorophenylpiperazine was administered intravenously at doses of 0.5, 1.5, and 3.0 mg/kg. Growth hormone and cortisol were increased significantly at all doses while prolactin was significantly increased only following administration of 3.0 mg/kg meta-chlorophenylpiperazine. Meta-chlorophenylpiperazine also produced behavioral alterations in most monkeys, including sedation, penile erection, and defecation. While the possibility that the observed neuroendocrine changes were a result of a general stress state secondary to behavioral alterations induced by meta-chlorophenylpiperazine exists, the fact that 0.5 mg/kg meta-chlorophenylpiperazine failed to consistently elicit behavioral responses, while the same dose produced maximal growth hormone and cortisol increases in each animal, suggests that the neuroendocrine responses are not dependent on manifest behavioral changes. Prolactin, growth hormone and behavioral responses to meta-chlorophenylpiperazine were completely blocked by pretreatment with the serotonin antagonist metergoline. Pretreatment with metergoline failed to entirely antagonize the cortisol response to meta-chlorophenylpiperazine. These data suggest that meta-chlorophenylpiperazine has prominent neuroendocrine and behavioral effects which are mediated, in part, by serotonergic mechanisms.

On the basis of these data from monkeys, we have begun studies with m-chlorophenylpiperazine in man. This agent should be of help in clarifying whether changes in serotonin receptor sensitivity or presynaptic serotonin system alterations are involved in the altered serotonergic responsivity we have observed using fenfluramine and α -tryptophan in depressed patients compared to controls and during treatment with psychoactive drugs.

Studies of serotonin and other indoleamines in monkey cerebrospinal fluid, which revealed large fluctuations in serotonin (but not of its major metabolite, 5-hydroxyindoleacetic acid), have been progressing slowly since the departure of Dr. Phil Taylor last year; this has required sending cerebrospinal fluid samples to Scotland for mass spectrometric analysis of indoleamines.

Significance to Biomedical Research and the Program of the Institute:

Serotonin and related indoleamines participate in the regulation of sleep, motor functions and several different hormones, including cortisol and prolactin. Abnormalities in these functions are found in depression and some other psychiatric disorders. We hope to use these plasma and cerebrospinal measurements as ways to examine the functional status of the brain serotonergic system, especially serotonin receptors, in comparing psychiatric patient groups and normal controls, and also to evaluate possible mechanisms of action of psychotherapeutic drugs in man.

Proposed Course:

Similar dose response relationships will be explored in man using meta-chlorophenylpiperazine and its antagonists in man, using neuroendocrine, behavioral and other physiological endpoints. Cerebrospinal fluid indoleamines and other substances will be studied in pinealectomized monkeys and in monkeys treated with a number of drugs which alter brain and/or pineal indoleamine release to clarify the source, regulation and consequences of changes in cerebrospinal fluid serotonin other indoleamines, and peptides related to the serotonergic systems.

Publications:

Garrick, N.A., Tamarkin, L., Taylor, P.L., Markey, S.P., and Murphy, D.L.: Light and propranolol suppress the nocturnal elevation of serotonin in the cerebrospinal fluid of rhesus monkeys. Science, 222: 474-476, 1983.

Aloi, J.A., Insel, T.R., Mueller, E.A., and Murphy, D.L.: Neuroendocrine and behavioral effects of m-chlorophenylpiperazine administration in rhesus monkeys. Life Sci. 34: 1325-1331, 1984.

Insel, T.R., and Goodwin, F.K.: The dexamethasone suppression test: Promises and problems of diagnostic laboratory tests in psychiatry. Hosp. Commun. Psychiatry, 34: 1131-1138, Dec. 1983.

Insel, T.R., and Goodwin, F.K.: The dexamethasone suppression test as a predictor of relapse. In Hirschfeld, R. (Ed.): The Clinical Utility of the Dexamethasone Suppression Test.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00338-04 CN

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Families of origin in obsessive-compulsive illness

PRINCIPAL INVESTIGATOR (List other professional persons who are participating investigators; list title, laboratory, and institute affiliation)

Carol F. Hoover, D.S.W.
CNB, NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Clinical Neuropharmacology Branch

SECTION

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

0.5

PROFESSIONAL:

0.4

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Family data were collected on 174 relatives of 10 severely obsessive-compulsive patients. No instances of obsessive-compulsive disorder were located, although 11.6% of first degree relatives had been hospitalized for other psychiatric illness. These rather isolated families had cultures which emphasized cleanliness and perfection, but other family members did not develop rituals or obsessive rationales as the patient did. Typically one or both parents involved in an unfulfilled marriage directed symbiotic needs toward the patient. Parents and offspring became trapped in an increasingly powerless struggle against symptoms which acted as a barrier to closeness, but which also prevented the patient from developing an autonomous existence. Parental symbiotic needs combined with perfectionist family styles, possibly superimposed on a constitutional vulnerability to psychiatric disturbance, appear to form a major contribution to obsessive-compulsive disorder.

Project Description

Objectives:

This study addressed the following questions: (1) the incidence of obsessive-compulsive disorder in relatives of late adolescent and adult patients with severe forms of the illness. (2) The psychosocial milieu presented in the families of origin. (3) Similarities or differences in personality, attitude, and coping styles between (a) patients, and (b) members of their nuclear families. (4) Patterns of family interaction, with special reference to parent/child and husband/wife relationships. (5) Observational comparisons between the immediate families of young schizophrenics and the families of severely obsessive-compulsive patients.

Methods Employed:

Family interview data were collected on 10 severely obsessive-compulsive patients, their 20 parents, 20 siblings, 3 offspring, and 131 second and third degree relatives. At least two relatives of each patient were interviewed, often over a period of months, and in most cases the families also participated in conjoint sessions.

Major Findings:

There were no relatives who suffered from classical obsessive-compulsive disorder, although other mental illnesses occurred in 11.6% of first-degree relatives. Four out of the 10 families had histories of hospitalization for mental illness among first-degree relatives, while two families had neurological or developmental abnormalities among their members. Six of the families were somewhat isolated from their communities, and seven included grandparents who were dominant figures in the family. All 10 of the families had cultures or life-styles which emphasized super-cleanliness, over-meticulousness and the like, which persisted through the generations. Although such a psycho-social milieu enhanced the development of obsessive-compulsive symptoms in an offspring, neither parents nor siblings adopted the patient's irrational explanations, nor did their standards of performance and cleanliness overwhelm them or become transformed into rituals. The patients' symptoms generally baffled and frightened their relatives, although parents were often bullied into indulging an offspring's bizarre practices.

There were severe conflicts within these nuclear families regarding intimacy and closeness. In seven of the existent marriages, relationships between the parents were unfulfilled, disappointing, strained, distant, or furiously argumentative, while two unions ended in divorce and another was interrupted by death. In nine out of the ten families, one or both parents concentrated more upon an offspring than upon each other, seeking an intense symbiotic involvement which was thwarted by the distancing obsessive-compulsive symptomatology in a son or daughter. The offspring simultaneously "used" the parental needs to achieve increased family power through

tantrums or other pressure tactics, yielding a parental helplessness and demoralization which contributed further to the developing illness. This engulfing symptomatology in turn made the patient helplessly dependent upon parental services, and unable to live apart from a caretaking family.

A speculation may be raised whether some genetic factor may contribute to the obsessional "culture" of over-meticulous habits observed in successive generations of these families, combining perhaps with elements in family relationships to produce obsessive-compulsive disorder in a vulnerable offspring.

Significance to Biomedical Research and the Program of the Institute:

This research has attempted to analyze and integrate family factors in the development of a severe and puzzling psychiatric disorder under investigation in the Institute, and to propose a comprehensive explanatory pattern which takes account of the family context in possible etiology.

Proposed Course:

This study is now completed and has been presented in published form.

Publications:

Hoover, C.F. and Insel, T.R.: Families of Origin in Obsessive-Compulsive Disorder. J. Nerv. Ment. Dis., 172: 207-215, 1984.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00339-03 CN

PERIOD COVERED
October 1, 1983 to September 30, 1984TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
The Neuropharmacology of Cognition

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Robert M. Cohen, Staff Physician CNB, NIMH

COOPERATING UNITS (if any)

LPP, NSB, NIMH
Enzor Research Foundation

LAB/BRANCH

SECTION
Clinical Neuropharmacology BranchINSTITUTE AND LOCATION
NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

2.3

PROFESSIONAL:

2.0

OTHER:

0.3

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Changes in cognition and emotion are a common concomitant of psychiatric and neuropsychiatric disorders. In prior work from this project we had demonstrated a close link between motivational state and memory performance of depressed patients, and had examined the effects of naloxone as a strategy to examine this linkage further. In normals, a dose of 2 mg/kg or higher of naloxone increased depression and anxiety ratings and induced decrements in memory performance. This year, we have tested the effects of 2 mg/kg of naloxone in depressed subjects, together with a second group of normals. Depressives manifested a more marked and subjectively more intense response to naloxone compared to normals. We have also examined the effects of naloxone in Alzheimer's patients. Preliminary data suggest that Alzheimer patients show behavioral and cognitive changes at lower naloxone doses than those effective in young normals. Two other drug strategies being actively investigated include the use of monoamine oxidase inhibitors and cholinergic antagonists in our efforts to continue to explore the links between cognition and emotion and its neuropharmacology.

Other Collaborative Professional Personnel Engaged on the Project:

D. L. Murphy, M.D.	Branch Chief	CNB NIMH
H. Weingartner, Ph.D.	Unit Chief	LPP NIMH
D. Pickar, M.D.	Section Chief	NSB NIMH
T. Sunderland, M.D.	Staff Physician	CNB NIMH
P. Tariot, M.D.	Staff Physician	CNB NIMH
M. R. Cohen, M.D.	Director	Enzor Research Foundation

Project Description:

Objectives: Memory deficits have been associated with a number of neurotransmitter pathways in specific disease states and in drug-induced states in normals. Although memory performance is a somewhat specialized form of behavior, the study of memory performance requires an understanding of the interaction of motivation, drive and attention with what may be the specific physiological events of remembering (the engram). Therefore, the study of cognition in depression and other neuropsychiatric illnesses may help to elucidate the specific mechanisms whereby the physiologic and biochemical substrates of these illnesses influence the central motivational state and its interaction with the specific processes involved in behavior in general, and in particular of memory. It is probably not coincidental that primary neuropsychiatric illnesses (SLE, Alzheimer's and Korsakoff's) are associated with mood disorders. Therefore, understanding the interrelationships between mood and cognition should aid in the treatment of some of the deficits in these disorders. The use of drugs to manipulate neurotransmitter systems in both patients and normals is viewed as a useful strategy in understanding these relationships.

Methods Employed:

Behavioral and Psychological Assessment: Diagnoses of major affective illness in each of the patients is made on the basis of Research Diagnostic Criteria with the aid of the Schedule for Affective Disorders and Schizophrenia. Degree of depression is measured by the nursing staff utilizing the Bunney-Hamburg 15-point ward rating scale, by physicians with the Hamilton rating scale, and by the subjects using the Beck Depression Inventory and the Profile of Mood States.

The diagnosis of dementia is based in part on the Hugh's Scale from Washington University which incorporates both Pfeiffer's short portable mental status questionnaire and the Blessed Scale in addition to other objective information obtained from an informant.

A number of tests of memory have been employed. These include measurements of short-term memory of items consisting of three consonants (e.g., MXP) working memory, recognition memory, and free recall memory predominantly. These tests are primarily verbal in nature, but in some instances visual memory is tested. Also a motor task measurement of sustained effort is employed wherein a subject's peak and subsequent sustained effort in squeezing a dynamometer is measured.

Biological Assessment: Plasma, platelets, urine and cerebrospinal fluid are collected for measurement of enzymes, levels of biogenic amines and their metabolites. The dexamethasone suppression test and the TRH stimulation tests are also used.

Major Findings:

As reported last year, memory performance in depressed subjects was strongly associated with decrements in motor performance and with severity of depression. Greatest depression-related impairment was found in those cognitive and motor tasks that required sustained effort.

In vivo experimental effects of opiate agonists and antagonists suggest that the endogenous opioid system is a modulator of the acquisition and retention of environmental events in animals. In man the opiate agonists appear to be important in the modulation of attention, pain and pleasure states. Although hormonal and nociceptive changes had been reported in man, no consistent alterations in cognition and mood had been observed following administration of the opiate antagonist naloxone. Using higher doses, only at 2 mg/kg or higher was memory and mood affected in young normals. In comparing 6 depressed patients with 8 volunteers in a subsequent study at 2 mg/kg naloxone, a significant effect was observed on objective rating scales of behavior and mood. These were greater in the depressed group, particularly in regard to paranoia and suspicion, and withdrawal-retardation. This was even more evident on the subjective rating scales and most pronounced in the subscales, tension-anxiety and depressive-dejection, although there were no significant differences between the groups in terms of physical symptomatology. Most recently, under the direction of Dr. Pierre Tariot, we have investigated the effects of varying doses of naloxone on a group of patients with both cognitive and emotional deficits, Alzheimer's patients. Even at 100 µg/kg, naloxone had significant behavioral effects on this group, particularly in the areas of hostility-suspiciousness, activation, irritability, restlessness and dysphoria. At this dose, 20-60 fold lower than the dose expected to induce changes in young normals, these patients also demonstrated an increase in irrelevant verbal associations.

In parallel to our work on the opiate system, Dr. Trey Sunderland is pursuing a similar strategy with respect to the cholinergic system. Preliminary data suggest that scopolamine, a cholinergic antagonist causes selective changes in mood and cognition in Alzheimer's patients and normals.

Significance to Biomedical Research and the Program of the Institute:

Cognitive changes frequently accompany the mood disturbances that characterize the affective disorders, with laboratory observations of depressed patients showing a positive correlation between the degree of impairment and the intensity of depression. So striking are these changes that they are frequently assigned a central role both in the etiology and treatment of depression. The finding of a close relationship between the decrements in performance in both motor and cognitive tasks in depression leads one to propose the parsimonious explanation of a single deficit in the central motivational state in depression. The methods developed for assessing the interplay of emotion and cognition in depression can be

usefully employed in studying other cognitive impaired populations to dissect the issues of motivation and reinforcement from other types of impairments (e.g., in Alzheimer's and Korsakoff's patients). Drug effects on cognition in these patients must be critically analyzed since motivation and effort may underlie cognitive changes which would otherwise appear to be direct effects on the memory processes themselves. Furthermore, these patients frequently have behavioral problems, including depression. The effects of drugs on these problems in this patient group have been inadequately studied.

Proposed Course:

We have been successful in gathering a population of subjects with a variety of neuropsychiatric disorders for a continuing in depth study of mood and the effects of neuropharmacologic challenge strategies on mood and cognition. As naloxone has recently been reported to alter cognition favorably in demented subjects, it was a natural choice given the background of our former studies with naloxone to pursue this in our study population. We need to test age-matched controls in this population to distinguish the effect of aging versus dementia on our naloxone findings. In addition, drugs that are specifically known to alter mood, e.g. antidepressants, are another logical choice with which to study the relationship of mood to cognition. We have already begun a study of the selective MAO B-inhibiting antidepressant, deprenyl, in this regard. Effects on cognition and the pattern of cognitive abnormalities observed in these subjects will be correlated with neuro-chemical findings. In this regard some patients do demonstrate behavioral changes on these drugs. We will continue our evaluation of anticholinergic antagonists in this population, as the cholinergic system has been implicated with respect to both mood and cognition and preliminary findings suggest interesting effects in both domains.

Publications:

Cohen, M.R., Cohen, R.M., Pickar, D., Sunderland, T., Mueller, E.A., and Murphy, D.L.: High dose naloxone in depression. Biol. Psychiatry, 19: 825-832, 1984.

Cohen, R.M., Sunderland, T., Aulakh, C.S.: Antidepressants in studies of cognitive dysfunction. Drug Dev. Res., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02218-01 CN

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biophysical approaches to medical therapeutics

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Jonathan L. Costa, Staff Physician, CNB, NIMH

COOPERATING UNITS (if any)

LMO, MB, LP, LPP, RO (NCI); FCRF; BEIB, DRS; NIAID; Lederle Laboratories; Hospital del Mar; Johns Hopkins Univ.; S-Cubed Corp., San Diego, CA

LAB/BRANCH

Clinical Neuropharmacology Branch

SECTION

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

3.0

PROFESSIONAL:

2.5

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Photoradiation therapy, a technique which involves administration of hematoporphyrin derivative plus exposure to laser light, produces an inflammatory response in atherosclerotic rabbit aortas. The technique may thus have some application in the treatment of atherosclerotic cardiovascular disease. Preliminary studies are also underway to determine whether or not the technique may be useful for treatment of metaplasia and neoplasia in human breast tissue. Rapidly alternating magnetic fields appear to retard the growth of primary breast carcinoma in rats, and are being examined for their effects on other systems and tumor types.

Other collaborative professional personnel engaged on the project:

R. Bonner	Biomedical Engineer	BEIB, DRS
E. Brown	Staff Physician	LCI, NIAID
M. Chirigos	Section Chief	FCRF
P. Ebert	Scientist	LMO, NCI
L. Fechter	Associate Professor	Johns Hopkins Univ.
P. Gullino	Laboratory Chief	LPP, NCI
K. Joiner	Section Chief	LCI, NIAID
J. Kwon-Chung	Section Chief	LCI, NIAID
C. Reichert	Staff Physician	LP, NCI
S. Schaffer	Director	Lederle Labs.
P. Smith	Biomedical Engineer	BEIB, DRS
D. Tschudy	Laboratory Chief	MV, NCI
A. Zabell	Staff Physician	RO, NCI
E. Mishuck	Chemist	S-Cubed Corp., San Diego, CA
F. Contreras	Surgeon	Hospital del Mar, Tijuana, Mexico

Project Description:

Objectives: To study the effects on cells and tissues of biophysical techniques, particularly lasers and alternating magnetic fields, and to explore ways in which these techniques may be useful in medical therapeutics.

Studies Implemented: (1) Use of photosensitization techniques to alter the growth and viability of leukemia L1210 cells in vitro; (2) preparation for in vivo studies of the utility of hematoporphyrin derivative and photoradiation therapy for the treatment of primary breast carcinoma and metaplastic foci; (3) exploration of the potential of hematoporphyrin derivative for photosensitization of atherosclerotic arteries; (4) studies of the effects of alternating magnetic fields on biological systems and on tumor growth.

Methods Employed:

1. General Preparative Procedures. Murine leukemia L1210 and MBL-2 cells were grown in serum-enriched medium and passaged after dilution. Various species of bacteria and fungi were incubated in broth media and allowed to grow to logarithmic or stationary phases. Atherosclerotic lesions were induced in the aortas of rabbits either by stripping the endothelium with a balloon catheter or by feeding the animals a diet rich in cholesterol. Primary carcinoma was induced in rats by gavage feeding of dimethylbenzanthracene, and Moloney leukemia by intraperitoneal inoculation with MBL-2 cells.

2. Effects of Hematoporphyrin Plus Light on L1210 Cells. Free-floating L1210 cells were incubated with hematoporphyrin or other compounds with a porphyrin ring. The amount of porphyrin taken up was measured by monitoring fluorescence after extraction with oxalic acid. Cells were exposed to light of varying wavelengths and the extent of cell killing quantitated by cell counts after the addition of trypan blue. Cell damage was also assessed by allowing the cells to grow in culture for periods up to one week before counting.

3. Photoradiation Therapy of Breast Neoplasia. Methods for irradiating breasts and breast tumors were developed utilizing a laser and model systems. Techniques for fixation and sampling of breast tissue after mastectomy were delineated with autopsy material.

4. Photoradiation Treatment of Atherosclerotic Lesions. Rabbits were given hematoporphyrin derivative intraperitoneally or intravenously. After varying periods of time, the aortas were irradiated intraluminally or extraluminally utilizing a dye-pumped laser operating at a wavelength of 631 nm. In vivo, rat livers and monkey skin were irradiated in a similar fashion after hematoporphyrin derivative administration. At intervals after photoirradiation, animals were sacrificed and tissue taken for autopsy. The uptake of hematoporphyrin derivative into atherosclerotic lesions and other tissues was evaluated by fluorescence microscopy of excised materials or

following the administration of radiolabelled compound. The histological appearance of irradiated and control tissues was evaluated after sectioning of frozen or fixed and embedded material.

5. Effects of Alternating Magnetic Fields on Biological Systems.

Cells, tissues, or whole animals were exposed to alternating magnetic fields of varying intensities. Parameters such as the viability and replication (growth) capacity of viruses, bacteria, fungi and cells were evaluated in exposed and unexposed material, utilizing plating assays or the uptake of radiolabelled compounds. During and after the periods when whole animals were exposed to the field, they were monitored for survival times or tumor growth rates. Fetuses produced following exposure of pregnant animals were sacrificed for histological and neurochemical analysis of brains.

Major Findings:

1. Effects of Hematoporphyrin Plus Light on L1210 Cells. L1210 cells accumulate appreciable amounts of hematoporphyrin, as well as a variety of other compounds with a porphyrin moiety (i.e. chlorophyll, cytochrome c, and horseradish microperoxidase). Approximately 10% of the accumulated material is retained when the cells are washed with serum-containing medium. Dibucaine, chloroquine and succinylacetone appear to enhance the uptake and retention of hematoporphyrin. After uptake of hematoporphyrin, L1210 cells are killed in a dose- and light-dependent manner when exposed to light at wavelengths corresponding to the absorption maxima of the hematoporphyrin in solution.

2. Photoradiation Therapy of Breast Neoplasia. Work with model systems suggests that it should be possible to photosensitize malignant cells in an entire breast prior to mastectomy. Procedures for evaluating the histopathological response of the tissue to irradiation have also been developed.

3. Photoradiation Treatment of Atherosclerotic Lesions. Large amounts of hematoporphyrin derivative accumulate specifically in the lipid-rich portions of atherosclerotic aortas, and are retained in this location for periods of up to one month after a single administration of the compound. Areas loaded with hematoporphyrin derivative can evidence an inflammatory reaction after exposure to sufficient quantities of light at 631 nm. Although fluorescence is not visible in the proliferative lesions associated with balloon stripping, these areas also appear to be photosensitized by the procedure.

4. Effects of Alternating Magnetic Fields on Biological Systems. An intense, rapidly alternating magnetic field (5 Tesla oscillating at 8 kiloHerz) appears to kill several species of bacteria which are associated with the spoilage of foods. However, many bacteria, fungi, and viruses pathogenic for mammals are unaffected by exposure to the same field. Although the membrane integrity and reproductive capacity of cells in culture are not materially altered, the growth rate of carcinogen-induced primary mammary carcinomas in rats appears to be reduced when the animals are exposed daily to the field. No teratogenic effects of such exposure have been observed.

Significance to Biomedical Research and the Program of the Institute:

Administration of hematoporphyrin derivative followed by exposure of selected tissues to laser light appears to be of potential value in two areas of medicine. First, since metaplastic or pre-neoplastic breast epithelium may be photosensitized and destroyed, the technique might be useful in the treatment of patients at increased risk for breast cancer (i.e., those with a strong family history, with documented carcinoma in the contralateral breast, or with fibrocystic disease). Second, provided that the arterial response to photosensitization involves resorption of lipid and fibrous accumulations, the same technique may be useful in the therapy of advanced atherosclerotic cardiovascular disease. A novel biophysical intervention, the application of an intense, alternating magnetic field to tumor-bearing animals, may be of some benefit in retarding the growth of primary mammary carcinomas.

Proposed Course:

Implement the planned study on the response to photoradiation therapy of breasts prior to mastectomy. Continue investigation of the tissue response of normal and atherosclerotic arteries to photoradiation therapy. Document the response to magnetic-field exposure of several in vivo tumor models, including mouse leukemia and rat primary breast carcinoma induced by a variety of carcinogens.

Publications:

Ebert P.S., Smith, P.D., Bonner, R.F., Hess, R.A., Costa, J.L. and Tschudy, D.P: Effect of defined wavelength and succinylacetone on the photo-inactivation of leukemia L1210 cells in vitro by hematoporphyrin. Photobiochemistry and Photobiophysics 6: 155-175, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02219-01 CN
PERIOD COVERED October 1, 1983 to September 30, 1984		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Animal models of anxiety		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Thomas R. Insel, Staff Physician, CNB, NIMH		
COOPERATING UNITS (if any) Johns Hopkins U.; U. of CO, Boulder; LCS; NICHD; NHLBI, NIADDK		
LAB/BRANCH Clinical Neuropharmacology Branch		
SECTION		
INSTITUTE AND LOCATION NIMH, NIH, Bethesda, Maryland 20205		
TOTAL MAN-YEARS: 2.2	PROFESSIONAL: 1.7	OTHER: 0.5
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>As an extension of our work with anxious and depressed patients, we have sought to develop pharmacologic models of anxiety in non-human primates. The benzodiazepine receptor active antagonist, <u>β-carboline-3-carboxylic acid-ethyl ester (β-CCE)</u> induced dose related increases in behavioral activation, plasma cortisol, and heart rate in rhesus monkeys. All of these effects were blocked by clinically relevant doses of diazepam but only selective parts of the β-CCE induced syndrome were blocked by clonidine or propranolol. The recently sequenced peptide <u>corticotropin releasing factor (CRF)</u> when given intraventricularly also was associated with an increase in plasma cortisol, but did not lead to an increase in behavioral activation. With CRF administration, increases in plasma catecholamines were evident only at very high doses. In an attempt to localize neural sites for CRF and possibly β-CCE actions, an <u>in vitro light microscopic autoradiographic method</u> for labelling specific CRF receptors in brain was developed. These receptors which are most densely localized in Lamina IV of the neocortex, the median eminence of the hypothalamus, and the amygdala, suggest a <u>circuit of brain structures</u> which may be involved in certain aspects of anxiety.</p>		

Other collaborative professional personnel engaged on the project:

D. L. Murphy	Chief	CNB	NIMH
S. M. Paul	Chief	NSB	NIMH
P. Skolnick	Section Chief		NIADDK
J. Crawley	Staff Scientist	NSR	NIMH
C. Pert	Section Chief	NSB	NIMH
M. Kuhar	Professor, Johns Hopkins U. Medical School		Baltimore, MD
S. Suomi	Chief	LCN	NICHD
J. Hill	Staff Fellow	LBEB	NIMH
T. Minor	Staff Fellow		Dept. of Psychology, U. of CO, Boulder
D. Jimerson	Section Chief	LCS	NIMH
D. Goldstein	Staff Scientist		NHLBI

Project Description:

Objectives. Although studies of patients with mental disorders have suggested a number of alterations in neuroendocrine or neurotransmitter function, it has not yet been possible to establish whether these altered functions are secondary manifestations of the mental disorder or actually etiologic in nature. The study of specific behavioral syndromes in animals may prove extremely important to clinical research by (1) modeling selective aspects of clinical disorders (2) permitting more invasive studies of brain function during the evocation of a particular set of behaviors (3) providing longitudinal, prospective analyses and (4) ultimately yielding a genetic approach from the study of individual differences. The challenge is to find models that are relevant to clinical problems and to choose dependent measures that are meaningful. This project, which is still within its first year, has had the following objectives: (a) to develop a pharmacologic model of anxiety in rhesus monkeys using the novel benzodiazepine receptor ligand β -carboline-3-carboxylic acid-ethylester; (b) to analyze the role of CRF as a possible anxiogenic agent in rhesus monkeys and (c) to map CRF receptors in brain in order to localize discrete brain structures relevant to the pathophysiology of anxiety.

Methods Employed:

In the studies evaluating a non-human primate model for anxiety, β -CCE was administered intravenously according to a randomized dosage schedule (0-500 μ g/kg). Behavior was rated in a double-blind manner using a scale developed from earlier β -CCE studies as well as from previously published studies of "anxiety-like" behavior in rhesus monkeys. Blood pressure and heart rate were monitored continuously using an ankle cuff. Plasma was obtained from an indwelling venous catheter for measurement of cortisol (by radioimmunoassay) and MHPG (by mass spectrometry, in collaboration with David Jimerson (LCS)). Following a dose-response study, the lowest dose necessary for behavioral and physiologic activation was administered in a subsequent study to assess the effects of pretreatment with each of three pharmacologically different anxiolytics (diazepam 0.5 mg/kg, clonidine 10 μ g/kg and propranolol 3 mg/kg).

Studies of CRF effects utilized an Ommaya reservoir for infusion of the peptide into the 4th ventricle. Plasma was sampled with an indwelling femoral venous cannula and assayed for catecholamines by David Goldstein (NHLBI) using an HPLC method.

CRF receptor studies included both in vitro light microscopic autoradiography and tissue homogenate binding assays. Autoradiographic studies require incubation of slide mounted frozen sections (8 μ) with 125 I-Nle 21-Tyr 32-CRF, a biologically active analogue of CRF (supplied by J. Rivier, Peptide Biol. Lab., Salk Institute, La Jolla, CA). Labelled sections are then apposed to radiation sensitive film to provide images of specific binding. Tissue homogenate assays utilize the identical isotope but are carried out on membrane suspensions rather than slide mounted sections.

Major Findings:

β -CCE administration resulted in significant dose related increases in behavioral agitation, plasma cortisol, and heart rate. Plasma MHPG did not change following even the highest dose of β -CCE. A clinically relevant dose of diazepam (0.5 mg/kg) blocked all of the effects of 100 μ g/kg of β -CCE, suggesting that (a) these effects were mediated via the benzodiazepine receptor, (b) the magnitude of these effects may be analogous to clinical anxiety and (c) the benzodiazepine receptor which had previously been implicated in the anxiolytic action of the benzodiazepines may also play a role in anxiogenesis. Further studies of CSF changes following β -CCE administration failed to demonstrate an increase in MHPG, suggesting that the drug does not activate the noradrenergic system. Nevertheless, administration of clonidine (10 μ g/kg), an α_2 -adrenergic agonist, prevented the cortisol and heart rate (though not behavioral activation) increase following β -CCE administration. Propranolol, a β adrenergic blocker, prevented the heart rate increase following β -CCE administration, but did not alter the cortisol or behavioral activation.

CRF (1-40 μ g/kg) intraventricularly did not induce a comparable anxiogenic syndrome in rhesus monkeys. Although animals became activated several hours following the highest dose of CRF (40 μ g/kg), the physiologic relevance of these behavioral effects was mitigated by the demonstration of profound increases in plasma cortisol at much lower doses (10 μ g/kg). Moreover, no change in plasma catecholamines could be demonstrated following any but the highest doses of CRF. The peptide was infused directly into the 4th ventricle, so noradrenergic centers in the pons should have been preferentially activated in this experiment. The failure to find an increase in plasma catecholamines is strong evidence against a primary role for CRF in the activation of noradrenergic pathways in primates.

CRF receptors have been demonstrated throughout the forebrain with greatest densities in Lamina IV of the neocortex, the outer band of the median eminence of the hypothalamus, and the lateral nucleus of the amygdala. Following the benzodiazepine receptor distribution, CRF receptors are also found in the cerebellum. As predicted from the in vivo primate studies, CRF receptors are not evident in the region of the locus ceruleus.

Significance to the Program of the Institute:

β -CCE offers a novel pharmacologic model of anxiety which can be used to electrophysiologically map discrete brain structures involved in selective anxiety syndromes. In addition, individual differences in the response to β -CCE will be an important focus for the new NIMH-NICHD collaborative project in Poolesville, Maryland.

CRF is a prominent brain peptide which has been implicated in the pathophysiology of both affective and anxiety disorders. This demonstration of specific receptors for CRF in mammalian brain (the first of its kind) provides important evidence that CRF in brain plays a physiologic role, possibly quite independent of its regulation of the pituitary release of ACTH.

Proposed Course:

β -CCE studies will continue (a) to study more comprehensively the changes in CSF amines and peptides following β -CCE administration (in collaboration with the Neuroscience Branch) (b) to measure individual differences in the response to β -CCE in animals that have been previously characterized as high and low responders to environmental stress (in collaboration with Steve Suomi of the NICHD and NIMH), and (c) to study direct, in vitro effects of benzodiazepine ligands on CRF receptor binding.

CRF in vivo studies will be extended with the intraventricular administration of selective antisera to this and related peptides for assessment of behavioral and physiologic effects.

CRF receptors will be measured in a series of animal models of psychiatric syndromes, including two rodent models of depression.

Publications:

Guttmacher, L.B., Murphy, D.L., and Insel, T.R.: Pharmacologic models of anxiety. Comp. Psychiatry, 24: 312-326, 1983.

Skolnick, P., Ninan, P., Insel, T., Crawley, J., and Paul, S.: A novel chemically induced model of human anxiety. Psychopathology, 17: (1)25-36, 1984.

Insel, T.R., Aloï, J.A., Goldstein, D., and Jimerson, D.C.: Plasma cortisol and catecholamine responses to intraventricular CRF administration. Life Sciences, 34: 1873-1878, 1984.

Insel, T.R., Ninan, P., Aloï, J., Jimerson, D., Skolnick, P., and Paul, S.: A novel benzodiazepine receptor mediated model of anxiety. Studies in non-human primates. Arch. Gen. Psychiatry (in press).

De Souza, E., Perrin, M., Insel, T.R., Rivier, J., Vale, W., and Kuhar, M.: Brain localization of CRF receptors by in vitro autoradiography. Science, (in press).

Insel, T.R., De Souza, E., Perrin, M., Rivier, J., Vale, W., and Kuhar, M.: CRF receptors in brain: Localization by autoradiography. Neurosci. Abstr. (in press).

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00446-15 CP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Inpatient Clinical Studies of Affective Illness

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: D. A. Sack Chief, Inpatient Services CPB/NIMH

Others: N. E. Rosenthal Chief, Outpatient Services CPB/NIMH
 W. B. Mendelson Chief, Unit on Sleep Studies CPB/NIMH
 B. L. Parry Clinical Associate CPB/NIMH
 S. P. James Clinical Associate CPB/NIMH
 J. A. Kline Clinical Social Worker CPB/NIMH

COOPERATING UNITS (if any)

LCS/NIMH

LAB/BRANCH

Clinical Psychobiology Branch

SECTION

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

The overall objective of this integrated group of research studies is a more comprehensive understanding of the pathophysiology of unipolar and bipolar affective disorder, as well as the more recently described seasonal affective disorder. The further development of our outpatient department has enabled us to greatly expand the investigative capacities of the inpatient unit. In contrast with previous years, the average admission for a patient with depression is short, approximately three weeks. During this time patients undergo a series of neuroendocrine, neurochemical and sleep and circadian studies which will enable us to further characterize their depressions. Patients with major affective disorder undergo a trial of partial sleep deprivation which may produce immediate therapeutic effects. Patients who are not improved are then started on drug therapy as part of double-blind investigations which are continued in the outpatient department. Shorter hospital stays have enabled us to increase the number of patients studied and to expedite therapy. Our ability to identify subgroups, predictors of response and to test specific hypotheses have all been expanded.

At the same time we have expanded our longitudinal studies of affective patients in two ways: (1) descriptive studies of rapid cycling manic depressives in entrained conditions; (2) studies of selected patients in conditions where they are isolated from time cues (temporal isolation).

The primary treatment modalities under investigation are: (1) environmental manipulations such as partial sleep deprivation and phototherapy; (2) tricyclic antidepressants showing specificity for given neurotransmitter systems; and (3) trials of euthyroid and hypermetabolic doses of thyroxine in rapid cycling manic depressives.

For project description and significance to biomedical research and to the program of the Institute see 1983 Annual Report. This project is being terminated because the activities of the clinical research unit are adequately described in other project reports that focus on specific aspects of affective illness and its treatment.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00450-10 CP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biological Rhythms in Affective Illness

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	D. A. Sack	Chief, Inpatient Services	CPB/NIMH
Others:	W. B. Mendelson	Chief, Unit on Sleep Studies	CPB/NIMH
	W. C. Duncan	Research Psychologist	CPR/NIMH
	N. E. Rosenthal	Chief, Outpatient Services	CPB/NIMH
	S. P. James	Clinical Associate	CPB/NIMH
	B. L. Parry	Clinical Associate	CPB/NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Psychology Branch

SECTION

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

1.0

PROFESSIONAL:

.5

OTHER:

.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unproduced type. Do not exceed the space provided.)

Basic research in biological rhythms can be related to affective illness with respect to (1) the involvement of disturbed circadian rhythms (24 hour cycles) in the pathophysiology of the illness and (2) the inherent cyclicality of the illness (itself a type of biological rhythm). Previous clinical investigations of many physiological and biochemical variables have shown that circadian rhythm phases, amplitudes and waveforms are abnormal in some depressives.

The apparent abnormalities in circadian rhythms seen in depressive disorders may arise from alterations in length of these rhythms (the free-running period), the interactions between rhythms, or in the effect which environmental cues (zeitgebers) have on these rhythms.

The timing of circadian oscillators cannot be measured directly in humans and indirect measures such as the secretory profiles of hormones, body temperature and EEG recorded sleep must suffice instead. External and internal factors called masking can influence the apparent circadian rhythms in these variables without affecting the biological clock or clocks. Physical activity, diet environmental lighting, differing sleep schedules and stress are common forms of masking. Differences in circadian rhythms between normals and depressives would arise from differences in masking rather than in the intrinsic properties of the biological clocks.

The present study is designed to compare the circadian rhythms of depressed patients and normal controls in conditions where the internal and external sources of masking have been controlled. Rhythms in cortisol, melatonin, TSH, neurotransmitter metabolites CHVA, MHPG, 5HIAA, core body temperature, sleep and activity will be assessed.

Project Description:

There is considerable evidence that biological rhythms are involved or are disturbed in affective illness. For example, the course of the illness is itself rhythmic: Depressive symptoms exhibit 24-hour patterns of variation in severity (the classical diurnal variation in mood), and depressions may recur cyclically every few days, weeks or months, or in some cases annually. The sleep-wake cycle is disturbed in depression and mania. Sleep disturbances include altered timing, amount and depth of sleep (these may be considered to represent changes in phase, waveform and amplitude of the sleep-wake cycle). Within depressive sleep the timing of REM sleep is abnormally advanced, possibly reflecting an advance in the phase position of the REM sleep propensity circadian rhythm. In some patients phase, amplitude and waveform of other circadian rhythms, such as rectal temperature, plasma cortisol and urinary potassium have been reported to be abnormal. The fact that experimental alterations in the timing of the sleep-wake cycle relative to other circadian rhythms leads to dramatic clinical state changes in many patients suggests that disturbances in the circadian system play an important role in the pathophysiology of the illness and are not simply epiphenomena.

Circadian rhythms in sleep, body temperature, hormones, and neurotransmitters have been previously studied in depressed patients and normal controls. Abnormalities in the amplitude (peak to trough difference) and the phase position (relative timing of these rhythms) have been described for these processes. The measurement of these rhythms is complicated by artifacts arising from symptoms which are central to depression. Diminished activity, weight loss, erratic eating schedules, and disturbed sleep schedules could account for some of the alterations in the circadian rhythms of depressed patients. These influences which alter the measurements of circadian rhythms without changing the intrinsic properties of the biological clock (or clocks) are called masking.

The purposes of this project are:

1. To replicate previously identified abnormalities in the circadian rhythms of depressed patients.
2. To determine the extent to which these abnormalities result from alterations in the circadian pacemaker or alternatively from masking caused by altered diet activity, or sleep in depressed patients.
3. To determine the relationship between circadian rhythm abnormalities and the antidepressant effects of sleep deprivation.

Methods:

Patients are included if they meet RDC criteria for major affective disorder (UP, BPI or BPII), and they are free of all psychotropic medications for at least three weeks.

Prior to beginning this study, subjects are adapted to an indwelling venous

catheter, rectal temperature probe, wrist activity monitor and psychological testing. On the baseline day specimens will be obtained every thirty minutes for cortisol and melatonin and every two hours for neurotransmitter metabolites via the indwelling catheter. The subject is ambulatory, in ordinary room lighting and on the regular ward diet. Psychological testing, mood ratings and a sleepiness rating are obtained hourly. Activity is measured by wrist activity monitor and core body temperature obtained by continuous monitoring of rectal temperature stored and recorded by computer every five minutes.

Beginning on the second day of the study room lighting is limited to 100 lux and an isocaloric liquid diet in equal hourly feedings is substituted for regular diet. Subjects are kept at bedrest and are continuously awake from the morning of the second day through noon of day three in order to control for differences arising from sleep and also to assess the clinical response to sleep deprivation. All previous measures are repeated on the second and third days.

Findings to date:

Thus far only four patients have completed the present study. Previous investigations revealed that:

1. Motor activity. Wrist motor activity levels are highly correlated with clinical state changes, with activity increased in mania and decreased in depression. Using a discrimination threshold, activity counts are highly correlated with wakefulness, so that the wrist activity monitor can be used to monitor the sleep-wake cycle.

2. Sleep EEG. The timing and amount of sleep changes cyclically with the illness. In many instances the switch from depression to mania is accompanied by the occurrence of one or several double-length, 48-hour sleep-wake cycles. These alternate sleepless nights may play an important role in the spontaneous depression-to-mania switch process since experimental sleep deprivation in such patients can induce depression-to-mania switches. Somewhat similar double-length sleep-wake cycles occur in normal persons when they are isolated from external time cues. Thus 48-hour sleep-wake cycles in patients may arise from a mechanism that is present, but latent, in the normal physiology of the human circadian system.

3. Rectal temperature. Compared with normal controls patients exhibit high night-to-night variability in the timing of their temperature minima. On many nights the temperature rhythm appears to be phase-advanced, as predicted by the phase-advance hypothesis of depression which attributes depressive REM sleep abnormalities to a phase-advance in the REM sleep propensity circadian rhythm.

Significance to Biomedical Research and to the Program of the Institute:

1. This study will extend our understanding of circadian rhythms in affective disorders. It will delineate the contribution of "masking" to these abnormalities.

2. It will provide additional evidence for extending or revising the present theoretical formulations regarding circadian rhythms in affective and sleep disorders.

3. It will establish whether a relationship exists between abnormalities described in neurotransmitters and their metabolites as measured in single time point studies of depressed patients and the circadian rhythms for these neurotransmitters.

4. It will determine whether the antidepressant effect to sleep deprivation is related to the circadian abnormalities seen in these patients.

Proposed Course:

A few additional patients will be sought for long-term, longitudinal studies. Much data, especially concerning the possible interrelationship between sleep EEG and temperature, remains to be analyzed.

Publications:

Wehr, T.A.: Biological rhythms and manic-depressive illness, in Ballenger, J.C., Post, R.M. (eds.), Neurobiology of the Mood Disorders, Williams and Wilkins, Baltimore/London, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02192-02 CP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Sleep in Psychiatric and Endocrine Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: W. B. Mendelson Chief, Unit on Sleep Studies CPB/NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Psychobiology Branch

SECTION

Unit on Sleep Studies

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

These studies represent collaborative projects with investigators interested in related issues which bear on sleep physiology. Included are a project showing decreased REM latency in panic-anxiety disorder patients, complementing a previous study here which showed the same process in obsessive-compulsive disorder patients. Following a long-term interest of the laboratory on sleep-related growth hormone secretion, patients with short stature and growth hormone neurosecretory dysfunction were found to have decreased number and amplitude of growth hormone pulses around the 24 hours, suggesting that there is a spectrum of GH regulatory dysfunction from absolute deficiency to irregularity of secretion. A variety of circadian rhythm studies continue, with evaluation of the sleep-waking aspects of longitudinal studies in patients with bipolar depressive illness and seasonal depression. In the latter group, seasonal variations in amounts of slow-wave sleep have been found.

For project description, other professional personnel, methods, findings to date and significance to biomedical research and to the program of the Institute, see 1983 Annual Report. The study of panic-anxiety patients has been completed, resulting in publication. Its main finding is that this patient group had a relatively short REM latency in comparison to controls. Other aspects of the project, which is primarily oriented to sleep, are described in the reports on the overall clinical projects: Z01 MH 00446-15 CP, Z01 MH 00450-10 CP, and Z01 MH 02201-02 CP.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02193-02 CP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical Studies of Insomnia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: W. B. Mendelson Chief, Unit on Sleep Studies CPB/NIMH

COOPERATING UNITS (if any)

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Clinical Psychobiology Branch

SECTION

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INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

0

PROFESSIONAL:

0

OTHER:

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CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The laboratory has had a longstanding interest in insomnia. Last year a major study of the effectiveness and hazards of benzodiazepine use in insomniacs was completed (Z01 MH 02193-01 CP). This year attention was directed to information processing and circadian rhythms in insomniacs. The data suggest that insomniacs may differ from non-complaining individuals in personality traits, cognitive style and temperature regulation. Insomniacs were found to have difficulty retrieving material already well known to them, although there may be no deficits in acquiring new material. They also had temperature curves similar to normals in phase, but about 0.4 F higher. Studies are currently under way to test the hypothesis that, because of heightened mental activity during sleep, insomniacs may perform differently in ability to perceive stimuli and acquire information while asleep.

For project description, other professional personnel, methods, findings to date and significance to biomedical research and to the program of the Institute, see 1983 Annual Report. This project resulted in one publication, and is now being expanded along the lines suggested in the Proposed Course described last year. So far, 8 insomniacs and 2 controls have completed the study, which involves arousal thresholds, classical conditioning during sleep, and other procedures. Data analysis is currently underway.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02194-02 CP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Anticonvulsant/proconvulsant effects of benzodiazepine receptor ligands

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: W. B. Mendelson

Chief, Unit on Sleep Studies

CPB/NIMH

COOPERATING UNITS (if any)

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Clinical Psychobiology Branch

SECTION

Unit on Sleep Studies

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

0

PROFESSIONAL:

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OTHER:

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CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Benzodiazepines are thought to have four general types of clinical effects: anxiolytic, muscle relaxant, hypnotic and anticonvulsant. One outgrowth of our previous work with benzodiazepine antagonists and sleep has been an interest in the role of GABA in the proconvulsant and anticonvulsant effects of drugs which bind to the benzodiazepine receptor. A current hypothesis suggested that drugs whose affinity to the receptor is decreased by GABA will be convulsants, while drugs whose affinity is enhanced will be anticonvulsants. This theory would predict that 3-carboethoxy-beta-carboline (beta-CCE), would have minimal or no proconvulsant properties, whereas the methoxy derivative (beta-CCM) would be a convulsant. Our laboratory demonstrated that when EEG criteria are used, beta-CCE will indeed induce electroencephalographic responses in rats, and our concurrent biochemical work indicated that the difference in effect can be explained by a faster rate of metabolism of beta-CCE. Similarly, biochemical studies in monkeys showed that the difference in relative resistance of rats to these seizures, and the relative ease of inducing seizures in squirrel monkeys, could be largely explained by rate of metabolism. These studies emphasize the importance of pharmacokinetic considerations as well as the role of GABA in drugs affecting seizure activity.

For project description, other professional personnel, cooperating units, methods, findings to date and significance to biomedical research and to the program of the Institute, see 1983 Annual Report. The project was completed, resulting in publication. The possible study of FG 7142 has been dropped, as our interests took us into other areas.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02195-02 CP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of the physiology and pharmacology of sleep

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: W. B. Mendelson

Chief, Unit on Sleep Studies

CPB/NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

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SECTION

Unit on Sleep Studies

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

 (a) Human subjects (b) Human tissues (c) Neither (a1) Minors (a2) Interviews

SUMMARY OF WORK (Use standard unproduced type. Do not exceed the space provided.)

The animal sleep laboratory has had an active year examining issues including the regulation of sleep, the pharmacology of anxiety and seizures, and approaches to growth hormone regulation. We previously reported that 3-hydroxy-methyl-beta-carboline, which binds to the benzodiazepine receptor and antagonizes the anxiolytic and anticonvulsant effects of diazepam, will induce dose-dependent increases in wakefulness and block the hypnotic actions of flurazepam. We have now given the tertiary butyl ester beta carboline (B-CCT), which is metabolized very slowly, and demonstrated a dose-dependent increase in wakefulness, the time course of which parallels receptor occupancy as measured in our laboratory. This provides further confirmatory evidence that the benzodiazepine receptor may play an important role in physiologic and pharmacologic sleep regulation. Another area of interest stems from the observation that drugs of very different pharmacologic classes may have behaviorally similar anxiolytic properties. In an effort to find some common mode of action, we hypothesized that the benzodiazepine receptor might play a role in the anxiolytic actions of barbiturates. Using Vogel's conflict test, we demonstrated that CGS 8216, a benzodiazepine antagonist, blocked the anxiolytic effects of pentobarbital. In other studies using the Vogel model, it was demonstrated that there are three types of effects on conflict procedures of drugs that bind to the benzodiazepine receptor: anticonflict effects of anxiolytic benzodiazepines, a pro-conflict effect of some beta carbolines, and antagonism of the other two effects by blockers such as CGS 8216 and RO 15-1788.

Following up several years of human work on the relationship of sleep to growth hormone (GH) secretion, we studied the effects of sleep deprivation on the ability of GH to stimulate tissue growth (measured by amount of activity of ornithine decarboxylase). Preliminary results indicate that sleep and exercise affect not only the secretion of GH, but also its effectiveness in stimulating growth.

For project description, other professional personnel, cooperating units, methods, findings to date and significance to biomedical research and to the program of the Institute, see 1983 Annual Report. This project has been completed, resulting in publication. The study listed as "1A" in Proposed Course in 1983 has been completed, indicating that CGS 8216, a benzodiazepine receptor blocker, did not block hypnotic effects of pentobarbital except at doses which by itself, had arousing effects. The other proposed studies have not been performed, as our interests turned to other aspects of receptor regulation.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02196-02 CP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Causes of the Delayed Sleep Phase Syndrome

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: T. A. Wehr Chief, Clinical Psychobiology Branch CPB/NIMH

Others: N. E. Rosenthal Chief, Outpatient Services CPB/NIMH
 W. B. Mendelson Chief, Unit on Sleep Studies CPB/NIMH
 D. A. Sack Chief, Inpatient Services CPB/NIMH
 S. P. James Clinical Associate CPB/NIMH

COOPERATING UNITS (if any)

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Clinical Psychobiology Branch

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INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

0

PROFESSIONAL:

0

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0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Delayed sleep phase syndrome (DSPS) is a type of sleep disorder characterized by marked difficulty falling asleep at night and marked difficulty waking up in the morning. When sleep occurs, it is normal in content and duration. Thus DSPS is a disorder of the timing, not the quality, of sleep. It is hypothesized that DSPS arises from a disturbance in the biological clock that regulates the timing of sleep. The normal human sleep-wake cycle is controlled by a biological clock that in the absence of external influences generates a 25-hour rhythm. The timing of this circadian rhythm ordinarily is controlled by periodic stimuli in the environment that have the properties of time cues (zeitgebers), so that it maintains (1) a period of 24 hours and (2) a characteristic phase relationship to the day-night cycle (i.e., sleep occurs every 24 hours, and mainly at night). Throughout biology light (e.g., sunrise, sunset) is the most important zeitgeber. In theory DSPS could be caused by (1) an abnormally slow intrinsic rhythm of the sleep-wake cycle pacemaker (e.g., 25.5-26 hours) or (2) an abnormal response to zeitgebers, such as light.

The purpose of this project is to investigate these two possible causes of DSPS: (1) the intrinsic rhythm of DSPS patients will be measured by asking them to live for at least two weeks in isolation from external time cues in special experimental rooms on the 4-West research unit. During the study motor activity and rectal temperature will be monitored continuously by computer. Circadian rhythm periods significantly longer than 25 hours would implicate a slow pacemaker as a cause of DSPS; (2) DSPS patients' hypothalamic sensitivity to light will be measured by investigating the degree to which nocturnal secretion of melatonin can be suppressed by varying intensities of light. Abnormal sensitivity to light would indirectly implicate altered sensitivity to zeitgebers as a cause of DSPS. Responses to light will also be evaluated using conventional optical and electrophysiological techniques.

For project description, methods, findings to date and significance to biomedical research and to the program of the Institute, see 1982-1983 Annual Report. This project has been temporarily suspended until adequate isolation facilities have been constructed.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02197-02 CP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Treatment of Delayed Sleep Phase Syndrome With Light

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: T. A. Wehr Chief, Clinical Psychobiology Branch CPB/NIMH

Others: W. B. Mendelson Chief, Unit on Sleep Studies CPB/NIMH

N. E. Rosenthal Chief, Outpatient Services CPB/NIMH

S. P. James Clinical Associate CPB/NIMH

COOPERATING UNITS (if any)

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TOTAL MAN-YEARS:

0

PROFESSIONAL:

0

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- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unproved type. Do not exceed the space provided.)

Delayed sleep phase syndrome (DSPS) is a sleep disorder characterized by difficulty falling asleep until 2 AM or later, normal sleep after sleep onset, and difficulty arising in the morning before 9 AM or later. DSPS is a disorder of the timing of sleep, not its quality, and as such is thought to arise from a disturbance in the biological clock in the brain that controls the sleep-wake cycle. Normally, the clock mechanism responds to periodic time cues in the environment (zeitgebers) in such a way that sleep occurs at night. In DSPS the timing of the clock mechanism is shifted later (delayed) so that sleep can only occur after 2 AM or later.

It is well documented in many species that light (e.g., dawn and dusk) is the zeitgeber that maintains the proper timing, or phase control, of biological clocks. Experiments are currently under way in this branch to establish whether or not light is an important zeitgeber in human beings (Project Z01 MH 02198-01 CP). The goal of this project is to determine whether or not delayed sleep phase syndrome can be treated using bright artificial light administered at a critical time of day. Based on animal studies we hypothesize that light given daily in the morning will gradually advance the timing of the sleep-wake cycle to a more nearly normal schedule in DSPS. We hypothesize that light administered in the evening will have no, or an opposite, effect. Morning and evening light will be administered to DSPS patients in a randomly sequenced cross-over design. Evening light appears to be a credible sham control treatment.

All patients entering the study will be carefully evaluated for the presence of other sleep, psychiatric and medical disorders. A special effort will be made to find possible predisposing or contributing factors, since little is known of the cause of DSPS. The daily sleep-wake cycle will be monitored longitudinally with wrist activity monitors developed at the NIMH.

For project description, methods, findings to date and significance to biomedical research and to the program of the Institute, see 1982-1983 Annual Report. This project has been temporarily suspended until equipment for precise and quantitative administration of light has been developed.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02198-02 CP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Effect of Light on Free-running Human Circadian Rhythms

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: T. A. Wehr Chief, Clinical Psychobiology Branch CPB/NIMH

Others: D. A. Sack Chief, Inpatient Services CPB/NIMH
 W. C. Duncan Research Psychologist CPB/NIMH
 N. E. Rosenthal Chief, Outpatient Services CPB/NIMH
 W. B. Mendelson Chief, Unit on Sleep Studies CPB/NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Psychobiology Branch

SECTION

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

0

PROFESSIONAL:

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- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unabbreviated type. Do not exceed the space provided.)

All species exhibit daily cycles in physiology and behavior called circadian rhythms. For example, in humans core body temperature oscillates between an afternoon high and a nighttime low. Circadian rhythms are generated by a pacemaker located in the hypothalamus. In isolation from external time cues (zeitgebers), the intrinsic period of this pacemaker is about 25 hours. Ordinarily the pacemaker responds to zeitgebers in such a way that it becomes entrained to the 24-hour solar day. In most species light (dawn, dusk) is the most important zeitgeber. The phase response to light of the circadian pacemaker is such that (1) its period becomes 24 hours and (2) it adopts a characteristic phase position relative to the zeitgeber (e.g., sleep occurs once every 24 hours and principally at night).

The effect of light pulses administered to animals free-running in constant conditions depends on when in the circadian cycle the pulse is presented. Pulses near subjective morning produce phase advances, near subjective evening delays, and during subjective daytime no effect. These responses can be used to generate a phase-response curve which shows direction and magnitude of phase shift as a function of subjective time of pulse presentation.

The purpose of this project is to generate a phase response curve to light for the human circadian system. Using suppression of pineal melatonin secretion as a marker, we previously found that human hypothalamus is relatively insensitive to light. Healthy subjects living for two weeks in constant dim light are exposed to a single 6-hour pulse of bright artificial light (3000 lux) administered at different subjective times in different subjects. Circadian rhythms are monitored longitudinally in rectal temperature, wrist motor activity, sleep EEG, and behavioral events. Shifts in these rhythms induced by light are recorded.

The normal human phase response curve is expected to prove valuable in understanding and treating circadian rhythm disturbances in depression, mania, insomnia (delayed sleep phase syndrome), jet lag and shift work.

For project description, methods, findings to date and significance to biomedical research and to the program of the Institute, see 1982-1983 Annual Report. This project has been temporarily suspended until isolation facilities with adequate acoustical insulation have been constructed.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02199-02 CP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Circadian Rhythms in Affective Disorder Patients Isolated From Time Cues

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: T. A. Wehr Chief, Clinical Psychobiology Branch CPB/NIMH

Others: D. A. Sack Chief, Inpatient Services CPB/NIMH
 W. C. Duncan Research Psychologist CPB/NIMH
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 N. E. Rosenthal Chief, Outpatient Services CPB/NIMH
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COOPERATING UNITS (if any)

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LAB BRANCH

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INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOXES

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unindented type. Do not exceed the space provided.)

Abnormalities of phase, amplitude and waveform of circadian rhythms have been reported in depression and in manic-depressive illness. Abnormalities of the circadian system are thought to play an important role in the illnesses because experimental manipulations of sleep and circadian rhythms are capable of altering clinical state.

The human circadian system has been mathematically modeled by a two process or two oscillator system. Depressive sleep abnormalities and the therapeutic effect of various sleep manipulations have also been modeled by changing certain parameters in these mathematical models, such as the intrinsic period of a circadian oscillator or the rates of accumulation and discharge of a sleep factor. We are testing these and other predictions of the models by studying circadian rhythms in patients while they live in isolation from all external time cues in special rooms on the 4-West research unit. Patients stay in the rooms while rectal temperature, wrist motor activity, sleep EEG and behavioral events are continuously monitored by computer. In these conditions circadian rhythms "free-run" according to their own intrinsic period, which is normally about 25 hours.

To date four affective patients have been studied for one month each. In one bipolar patient who switched from depression to mania shortly after beginning the experiment, the free-running period was abnormally short (less than 24 hours). Such an abnormally fast circadian pacemaker could explain phase-advanced circadian rhythms under conditions of entrainment to external time cues. A manic patient showed internal desynchronization of sleep and temperature circadian rhythms with a sleep-wake cycle period of 17 hours. The circadian period in two other cases was normal. All cases showed markedly abnormal ratios of sleep to wakefulness during the experiments, indicating that changes in timing and amount of sleep in depression are fundamentally endogenous and do not depend on some interaction with the environment.

For project description, methods, findings to date and significance to biomedical research and to the program of the Institute, see 1982-1983 Annual Report. This project has been temporarily suspended until isolation facilities with adequate acoustical insulation have been constructed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02200-02 CP
PERIOD COVERED October 1, 1983 to September 30, 1984		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Light Suppression of Nocturnal Human Melatonin Secretion		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	T. A. Wehr	Chief, Clinical Psychobiology Branch CPB/NIMH
Others:	S. P. James	Clinical Associate CPB/NIMH
	B. L. Parry	Clinical Associate CPB/NIMH
	D. A. Sack	Chief, Inpatient Services CPB/NIMH
	N. E. Rosenthal	Chief, Outpatient Services CPB/NIMH
COOPERATING UNITS (if any)		
LAB/BRANCH Clinical Psychobiology Branch		
SECTION		
INSTITUTE AND LOCATION NIMH, NIH, Bethesda, Maryland 20205		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0
CHECK APPROPRIATE BOX(ES)		
<input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither		
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Melatonin (MT) is secreted by the pineal gland (PG) almost exclusively at night. Our previous work has shown that MT is present in humans and that its secretion can be suppressed with bright artificial light (>2000 lux). This effect of light is presumed to be mediated by neural pathways connecting the retina to the PG via the hypothalamus. At the hypothalamic level the effect of light is thought to be mediated by nicotinic cholinergic receptors. Suppression of MT is presently the only index of hypothalamic sensitivity to light. We have shown that humans have a high threshold for light-MT effects compared with experimental animals. Ordinary artificial light, for example, is ineffective in humans.</p> <p>Abnormal hypothalamic sensitivity to light may be an important trait and pathogenic mechanism in manic-depressive illness (Project Z01 MH 02199-01 CP), seasonal affective disorder (Projects Z01 MH 02205-01 CP and Z01 MH 02206-01 CP) and delayed sleep phase syndrome (Projects Z01 MH 02196-01 CP and Z01 MH 02197-01 CP).</p> <p>The purpose of this project is (1) to standardize the light-MT suppression test (LMST), (2) to identify sources of variance in the LMST (age, sex, prior sleep or waking, prior exposure to light, etc.), and (3) to investigate hypothalamic sensitivity to light using the LMST in the disorders outlined above.</p> <p>We will standardize the administration of light using high-pressure xenon and xenon-mercury lamps in association with ultraviolet and infrared filters modifying light projected into a ganzfeld dome. Intensities of light can be varied by using neutral density filters. Blood samples will be obtained before and after light administration. Using these methods a fluence-response curve for the LMST will be generated with the degree of MT suppression expressed as a function of log light intensity (watts/cm²).</p>		
247		

For project description, methods, findings to date and significance to biomedical research and to the program of the Institute, see 1982-1983 Annual Report. This project has been temporarily suspended until equipment for precise and quantitative administration of light has been developed.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02201-02 CP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Early Versus Late Partial Sleep Deprivation in the Treatment of Depression

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: D. A. Sack Chief, Inpatient Services CPB/NIMH

Others: T. A. Wehr Chief, Clinical Psychobiology Branch CPB/NIMH
 N. E. Rosenthal Chief, Outpatient Services CPB/NIMH
 B. L. Parry Clinical Associate CPB/NIMH
 W. B. Mendelson Chief, Unit on Sleep Studies CPB/NIMH
 S. P. James Clinical Associate CPB/NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Psychobiology Branch

SECTION

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

2.0

PROFESSIONAL:

1.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Various manipulations of the sleep wake cycle have antidepressant effects in endogenously depressed patients. Total sleep deprivation and partial sleep deprivation in the second half of the night induce remissions during the following day. Also, advancing the sleep period several hours earlier than its usual time without a decrease in the length of the sleep period (phase-advance of the sleep wake cycle) also may induce clinical remissions.

Some studies show that the timing of various circadian rhythms and REM sleep appears to be shifted to an abnormally early time in depression. In patients with phase-advanced circadian rhythms, both the phase-advance treatment and partial sleep deprivation in the second half of the night, shifts sleep earlier, restoring a more normal relationship between sleep and the other circadian rhythms. If the timing of sleep and not the duration is the critical factor in the sleep deprivation response, then partial sleep deprivation in the first half of the night should not have therapeutic effects.

As part of a continuing study, we have examined the relative efficacy of partial sleep deprivation (PSD) in the first half of the night (E = early) and PSD in the second half of the night (L = late) in a randomized crossover design. All depressed patients underwent baseline sleep, temperature, activity, and 24 hour urinary collections for norepinephrine, serotonin and their metabolites in order to investigate the relationship between the sleep deprivation response and the current biochemical and neuroendocrine hypotheses of depression.

Of 18 drug free depressed patients (11 BP, 7 UP) 10 responded to PSD. All 10 improved on the PSD-L condition. None responded when kept awake in the first half of the night. Results of this study support the hypothesis that the internal phase relationship between sleep and other circadian rhythms is critical to the antidepressant response of PSD. In addition the response to PSD was not restricted to the first night. Improvement was sustained through a second night of PSD and a night of recovery sleep.

Project Description:

The timing of various circadian rhythms appears to be shifted to an abnormally early time in depression, raising the possibility that the timing of sleep relative to circadian rhythms (their internal phase relationship) is a pathogenic factor in affective illness. The antidepressant effects of partial sleep deprivation may, therefore, depend on the time at which sleep occurs. The objectives of this study are:

- 1) To determine the relative efficacy of sleep deprivation in the first half versus the second half of the night.
- 2) To describe the diagnostic, biochemical neuroendocrine and psychophysiological predictors of the sleep deprivation response.
- 3) To determine the effects of sleep deprivation on neurotransmitters whose function is thought to mediate other antidepressant responses.

Methods:

Patients were included if they met RDC criteria for a major affective disorder, and had been free of all psychotropic medications for at least two weeks. All subjects underwent a baseline evaluation which included: 1) EEG recorded sleep, 2) 24 hour rectal temperature monitoring, 3) 24 hour urine collection for norepinephrine and its metabolites and urinary free cortisol. EEG recorded sleep and 24 hour urine collections were repeated daily throughout the study. Following two baseline days each subject was randomized to partial sleep deprivation (PSD) in the first half of the night (sleeping from 2 a.m. to 7 a.m.) or PSD in the second half of the night (sleeping from 9 p.m. to 2 a.m.). Patients were kept on the PSD schedule for two successive nights and then returned to a normal sleep schedule (11 p.m. to 7 a.m.). We anticipated that our patients might experience differing degrees of difficulty in sleeping on the two treatment schedules. In order to control for the differences in sleep duration resulting from the experimental conditions we allotted up an additional hour for patients to fall asleep on each condition. Sleep onset was determined by EEG and patients were allowed to sleep up to five hours. Thus the time spent in bed for the two treatments were 8 p.m. to 2 a.m. for PSD-L and 2 a.m. to 8 a.m. for PSD-E. In no case were patients allowed to sleep past 2 a.m. (on PSD-L) or 8 a.m. (on PSD-E) even if the total sleep was less than 5 hours.

Improvement in mood was assessed on a nurses global assessment scale and a visual analogue self rating scale (100 mm line) obtained every two hours while awake through all five days of the study. In addition a standardized video taped interview was performed every four hours during the same period to be used for "blind" ratings.

Patients who relapsed following several recovery nights sleep, were crossed over to the other PSD condition after the baseline studies had been repeated.

Findings:

Eighteen subjects have been studied, sixteen of whom completed the crossover design. Ten subjects improved when PSD occurred in the second half of the night. Clinical improvement was sustained on the day following a second night of PSD-L, and a single night of recovery sleep.

Baseline EEG sleep recordings revealed differences between responders and non-responders. Responders had significantly shorter REM latencies and longer first REM periods. Those findings are consistent with a phase advance of the oscillator which controls REM sleep in the responder group.

Despite our attempts to control sleep duration on the two treatment conditions, patients slept on the average one hour less on the PSD-L condition. Thus it is possible that shorter sleep duration may have contributed to response differences.

REM sleep was significantly different on the treatment nights for the two experimental conditions. REM sleep duration was reduced by almost 50% on the PSD-L condition but was unchanged from baseline on the PSD-E condition. Previous studies have demonstrated that selective REM deprivation has antidepressant effects but the time course of the response (two - three weeks) is considerably slower than in our PSD experiment.

Significance to Biomedical Research and to the Program of the Institute:

1. Our findings indicate that PSD in the first half of the night can be a plausible sham procedure in future clinical studies. This is important because previous studies of sleep deprivation were hampered by the lack of an adequate control.

2. Sleep deprivation is one of three effective treatments for severe depression (antidepressants and ECT being the other two) but its clinical application has been limited by the brevity of its response. It appears from this limited sample that PSD can produce marked and sustained effects and that its clinical application and its mechanism of action require further investigation.

3. Partial sleep deprivation provides a clinical bridge between circadian and biochemical formulations of depression. The effects of PSD on neurotransmitter function may provide important insights into the pathophysiology of depression. Studies of neuronal function during the second half of the night may suggest new pharmacologic models for antidepressant medications.

4. This experiment provides further evidence that the timing of sleep is critical to the maintenance of the depressive syndrome in certain individuals. It appears that the second half of the night corresponds to a "critical phase" when sleep or wakefulness exerts a powerful effect on the clinical state of depressed patients. This antidepressant effect is most likely to occur in patients with short REM latencies and long first REM periods, as would be predicted by a phase-advance hypothesis. This suggests

that the pathophysiology of depression can only be understood when patients are studied from 2 a.m. to 7 a.m., a time when they have seldom been studied in the past.

Proposed Course:

In the following year we intend to alter our experimental design to enable us to more effectively control for sleep duration as variable in the PSD response.

A second question which we hope to answer is whether repeated PSD over an extended period of time would have a sustained antidepressant effect similar to that seen with tricyclic antidepressants and MAO inhibitors.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02202-02 CP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical Features of Seasonal Affective Disorder (SAD)

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: N. E. Rosenthal Chief, Outpatient Services CPB/NIMH

Others: D. A. Sack Chief, Inpatient Services CPB/NIMH
 S. P. James Clinical Associate CPB/NIMH
 B. L. Parry Clinical Associate CPB/NIMH
 W. B. Mendelson Chief, Unit on Sleep Studies CPB/NIMH
 T. A. Wehr Chief, Clinical Psychobiology Branch CPB/NIMH

COOPERATING UNITS (if any)

BPB/NIMH
 University of Pennsylvania
 NINCDS

LAB/BRANCH

Clinical Psychobiology Branch

SECTION

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

1.8

PROFESSIONAL:

0.6

OTHER:

1.2

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Epidemiological studies have shown a strong relationship between the incidence of suicides, affective episodes and the seasons. In order to understand this association, we have studied patients who regularly become depressed at a certain time of year. Most of these patients become depressed in winter and recover or become hypomanic in spring or summer. We have studied over 100 such patients with Seasonal Affective Disorder (SAD). Most are women with an onset of illness in their twenties. During depressions they become lethargic, overeat, oversleep, gain weight and withdraw from friends and family. Although they rarely require hospitalization and hold their jobs, their level of functioning deteriorates. Most SAD patients note that their depressions improve when they travel south in the winter.

Although the above pattern of SAD is the one most frequently encountered, an analysis of 602 questionnaires revealed that other patterns, including regular summer and spring depressions also occur.

Children as well as adults may be affected by SAD and when this occurs, the symptoms may be somewhat different than in the case of adults.

Anecdotal reports suggest that after winter light treatment, the course of the condition during subsequent seasons may be altered.

Ongoing studies involve: (1) systematic follow-up of patients who have been treated with light therapy for their winter depressions; (2) studies of children and adolescents with SAD; (3) further delineation of the relationship between climatic variables and mood, sleep and behavior.

Project Description:

There is a well established association between seasonal changes and the incidence of affective episodes and suicides in the population. Seasonal variations in several neurotransmitters and hormones considered to be relevant to the pathophysiology of depression have been described. Although these studies have shown correlations between mood states and the seasons, their design has not allowed for a clear understanding of the mechanism of these seasonal influences.

By studying the clinical characteristics of a group of patients with a history of regularly occurring seasonal affective shifts, we hope to improve our understanding of the way in which physical environmental changes affect vulnerable individuals.

Methods:

Our group has described the syndrome of Seasonal Affective Disorder (SAD) over the past three years. Patients are predominantly women with an onset of their disorder in the twenties. They typically become depressed in the fall and remit in the spring. Most are bipolar, especially bipolar II with hypomania occurring in spring or summer. About a third of all patients have had no previous treatment. A high percentage of first-degree relatives have a history of affective disorder. Depressions were generally characterized by depressed mood, hypersomnia, hyperphagia, weight gain, carbohydrate craving, low energy level and impaired functioning.

We have followed five children with a history of symptoms reminiscent of SAD in order to define the manifestations of this condition in younger people.

We have analyzed the screening questionnaire of all people who felt they might have a seasonal mood problem have divided these questionnaires into clinical subgroups and have analyzed the differences between subgroups.

Findings to date:

1. During the past year we encountered five adolescents (aged 13 to 16 years), who had reported symptoms of SAD in previous years. Three of these patients are children of adults in our program. Common symptoms include: (1) impaired school performance in fall and winter, (2) schoolwork and other tasks require far greater effort to achieve the same results, and (3) the perception on the part of the children that teachers and parents are making unfair demands, even though the demands themselves remained unchanged.

2. Our analysis of 602 screening questionnaires showed a preponderance of fall-winter depressions (53%) although some described regular summer (2.7%) or spring (0.9%) depressions. Some respondents had suffered from non-seasonal major affective disorder (17.3%) and others could not be assigned to any particular diagnostic category (6%). When we compared the SAD, non-seasonal affective and "undiagnosed" groups, we found that the SAD patients were more preponderantly women and more frequently reported overeating than did the other two groups ($p < .05$). This suggests that these clinical features may be

specific to SAD and not merely a function of the population who responded to our recruitment strategy.

Significance to Biomedical Research and to the Program of the Institute:

1. The description of SAD has focused attention on a group of patients whose suffering, in many cases, went largely unrecognized. To judge by the number of responses (about 4,000 from all parts of the country), the problem

is not uncommon. Psychiatrists and other physicians should be more aware of the syndrome, especially because it is so easily treated.

2. The careful clinical descriptions we have provided serve as a valuable basis for researchers interested in studying the effect of the climate on mood and behavior. These highly sensitive and reactive individuals are ideal subjects for such studies.

3. The observation that children may be affected by this condition may help clinicians and teachers assist children with seasonal emotional and learning difficulties and, by catching the problem early, minimize its impact on development.

4. By showing that there is a variety of patterns of seasonal mood problems, we open up the possibility of exploring different mechanisms by means of which the environment may affect vulnerable individuals.

Proposed Course:

Besides following promising avenues of research currently underway, we are planning to extend this study in the following ways:

1. We are in the process of developing a computer-scorable instrument for determining by self-report the degree to which individuals are affected by the seasons. We plan to use this instrument to evaluate the prevalence of seasonal mood problems in populations including:

- (a) family members of bipolar patients (in conjunction with Drs. Elliot Gershon and John Nurnberger).
- (b) the general population. We are exploring the possibility of studying this in collaboration with investigators at other centers.

2. We are planning to study systematically the course of patients who have been treated in our program. We are specifically interested in whether light treatment has a long-term effect on the course of a patient's illness, a question of both practical and theoretical importance.

3. We are planning to extend our study of SAD in children by:

- (a) questioning the children of our adult SAD patients, who are at

high risk for this condition.

- (b) recruiting and studying a population of children with this problem.

Both of these studies are going to be done in collaboration with Dr. William Sonis. The latter study will be undertaken predominantly at the University of Minnesota, to which Dr. Sonis will be relocating.

Publications:

Rosenthal, N.E., Sack, D.A., Gillin, J.C., Lewy, A.J., Goodwin, F.K., Davenport, Y., Mueller, P.S., Newsome, D.A., Wehr, T.A. Seasonal Affective Disorder: a description of the syndrome and preliminary findings with light therapy. Arch Gen Psychiatry. 41:72-80, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
 Z01 MH 02203-02 CP

PERIOD COVERED
 October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
 Sleep, Temperature and Activity Changes in Women With Premenstrual Syndrome

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)
 PI: T. A. Wehr Chief, Clinical Psychobiology Branch CPB/NIMH

Others: B. L. Parry Clinical Associate CPB/NIMH
 W. B. Mendelson Chief, Unit on Sleep Studies CPB/NIMH
 N. E. Rosenthal Chief, Outpatient Studies CPB/NIMH
 D. A. Sack Chief, Inpatient Services CPB/NIMH
 S. P. James Clinical Associate CPB/NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
 Clinical Psychobiology Branch

SECTION

INSTITUTE AND LOCATION
 NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	0.5	PROFESSIONAL:	0.25	OTHER:	0.25
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CHECK APPROPRIATE BOX(ES)
 (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The symptoms of premenstrual syndrome (PMS) consist of mood, cognitive, and behavioral disturbances occurring in the premenstrual phase of the cycle. They may become severe enough to cause suicidal depression or psychosis. Objective physiologic parameters that correlate with the subjective symptoms of PMS need to be identified in order to delineate this syndrome further and possibly to suggest better forms of treatment. First, this study will examine sleep, temperature and activity changes across the menstrual cycle in ten women with moderate to severe premenstrual syndrome and in normal volunteers. One month of baseline activity recording, objective ratings and self-ratings of sleep, mood, and energy will be obtained. Subjects will then be admitted to the hospital where they will undergo sleep EEG and temperature recordings two nights a week for the duration of one menstrual cycle.

Premenstrual syndrome may represent a variant of affective disorder. Therefore, treatment modalities found to be effective in the major affective disorders may be useful in treating patients with PMS. For example, sleep deprivation which induces transient remissions in affective disorder may do the same in PMS. Furthermore, sleep deprivation lowers prolactin, and hyperprolactinemia has been associated with mood disturbances in patients with PMS. Therefore, the effects of sleep deprivation will be investigated in these patients. Prolonged intense light exposure alleviates symptoms in patients with seasonal affective disorder. Since symptoms of SAD and PMS are similar, prolonged intense light exposure will be evaluated as a possible treatment for PMS.

Results of sleep deprivation and light treatment experiments may increase our understanding of the pathophysiological mechanisms of PMS and the relationship between PMS and affective disorders.

Project Description:

A high percentage of women have mood, cognitive and neurovegetative disturbances associated with their menstrual cycle. In particular, in the premenstrual phase they report such symptoms as depression, anxiety, irritability, difficulty concentrating, as well as sleep, appetite and energy disturbances. These symptoms often become severe enough to disrupt normal functioning in work and interpersonal relationships in some, and have resulted in psychosis and suicidal depressions in others. There is some question whether this syndrome may represent a variant of an affective disorder. In order to delineate further premenstrual syndrome (PMS), to understand better its physiologic basis, and to provide potentially better forms of treatment, objective biological variables need to be followed across the menstrual cycle that may then be correlated with subjective mood and behavioral changes. Neuroendocrine parameters have been the focus of previous and ongoing studies. This study will first focus on sleep, temperature and activity changes across the menstrual cycle. Nonpharmacologic strategies for treatment intervention using sleep deprivation or exposure to intense light will be used. Patients with affective disorder frequently show characteristic patterns of change in their sleep, temperature and activity rhythms. Also, sleep deprivation has been found to be affective in ameliorating depressive symptoms in many patients with major affective disorder. By studying these same parameters in patients with premenstrual syndrome, a better understanding of the relationship of PMS to affective disorders may be achieved. Furthermore, sleep and circadian rhythm disturbances appear to play a key role in the pathophysiology of affective disorder. This study will help to determine whether the same is true of PMS.

One major neuroendocrine theory of PMS proposes premenstrual hyperprolactinemia as etiologic in the development of mood symptoms at that time; since sleep deprivation lowers prolactin, sleep depriving women in the premenstrual phase of their cycle may help alleviate symptomatology at that time. In any case, sleep deprivation has antidepressant effects in non-PMS depressives. The time-limited effects of sleep deprivation have restricted its use as a clinical treatment modality in patients with affective disorder. However, since premenstrual syndrome is itself time limited, sleep deprivation, if effective, may be a useful clinical mode of treatment. Anecdotally, women with seasonal affective disorder (SAD) and PMS have reported improvement in their premenstrual symptomatology when treated with light. Therefore, extending the photoperiod by exposure to high intensity light will be tried also in this study (without causing sleep deprivation) to determine its efficacy in relieving symptoms of PMS.

Methods:

Ten women with moderate to severe premenstrual syndrome will be evaluated with screening forms, daily rating forms and personal interviews. Those who meet DSM III criteria for major affective disorder in the premenstrual phase of their cycle only, will be included in the study, as well as age-matched controls. One month of baseline activity recording and self-rating scales will be obtained before admitting the patients to the hospital where sleep and temperature recordings will be obtained two nights per week for the duration of one menstrual

cycle. In the third month, sleep deprivation or extended light exposure will be instituted in the premenstrual phase. Blood samples for estrogen, progesterone, and prolactin will be obtained weekly to document ovulation and to correlate behavioral and neuroendocrine events. More conventional modes of treatment for PMS will then be instituted in the event that the aforementioned experimental treatments are not effective.

Findings to date:

The study is currently in its preliminary stages, so there are no findings to date. Sleep and activity changes across the menstrual cycle are suggested by previous reports in the literature. Ten subjects have been run, five patients and five age-matched controls. There is some suggestion of efficacy with use of lights and sleep deprivation, but this needs to be substantiated with further trials planned for the upcoming year.

Significance to Biomedical Research and to the Program of the Institute:

PMS is responsible for morbidity in 40-60% of the female population. No objective physiologic manifestations of PMS have been identified that could be used to follow its course and to measure its response to treatment. Furthermore, current pharmacologic treatment modalities have not been consistently effective and are fraught with side effects. Changes in sleep, temperature and activity across the menstrual cycle may prove to be useful physiological markers of PMS symptoms; also, sleep deprivation or light therapy may be an effective non-pharmacologic treatment. On a conceptual level, the study may elucidate the role of changes in sleep, temperature, activity, and biological clocks in the pathophysiology of PMS.

Proposed Course:

Should total sleep deprivation be an effective clinical treatment of PMS, more specific alterations of sleep schedules such as partial sleep deprivation or shifting the time of sleep will be applied. Should the clinical effects of sleep deprivation be correlated with the lowering of serum prolactin, then a series of pharmacological studies will be done to determine whether or not prolactin inhibition mediates the sleep deprivation response. For example, if nighttime infusions of L-DOPA, which lowers prolactin, are given when subjects are asleep, will this also produce the same antidepressant response as sleep deprivation? Also, if TRH is given acutely, or neuroleptics chronically, to increase prolactin while patients are sleep deprived, will this counteract the antidepressant response of sleep deprivation? Should "light therapy" be effective in ameliorating premenstrual symptoms, a study of women's sensitivity to light across the menstrual cycle will be undertaken to determine whether it might be a state or trait marker.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02204-02 CP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of Sleep as a Circadian Rhythm

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: W. B. Mendelson

Chief, Unit on Sleep Studies

CPB/NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Psychobiology Branch

SECTION

Unit on Sleep Studies

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Unit has conducted a series of projects to elucidate the rhythmic aspects of sleep, and has also participated in broader studies of biological rhythms in depressive illness. In order to determine if the circadian rhythms of temperature and sleep represent "the hands of the clock" or whether they interact on some more fundamental level, studies of sleep are being performed on subjects wearing a "space suit" on loan from NASA. Using control systems developed here, the suit can virtually abolish the circadian rhythm of temperature. In another project normals and depressed patients are placed on a 1 hour dark/2 hour light schedule to enhance the rhythmic aspects of REM sleep and determine if depressives are phase advanced in this measure.

For project description, other professional personnel, methods, findings to date and significance to biomedical research and to the program of the Institute, see 1983 Annual Report. Because of a variety of improvements and refinements of technique, this project is considered completed; one aspect of this work is now in new project Z01 MH 02226-01 CP, in the 1984 Annual Report.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02205-02 CP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Effects of Light Interventions in Seasonal Affective Disorder (SAD)

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: N. E. Rosenthal Chief, Outpatient Services CPB/NIMH

Others: D. A. Sack Chief, Inpatient Services CPB/NIMH
 S. P. James Clinical Associate CPB/NIMH
 B. L. Parry Clinical Associate CPB/NIMH
 W. B. Mendelson Chief, Unit on Sleep Studies CPB/NIMH
 T. A. Wehr Chief, Clinical Psychobiology Branch CPB/NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Psychobiology Branch

SECTION

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

1.2

PROFESSIONAL:

0.4

OTHER:

0.8

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have previously shown in three separate groups of patients with Seasonal Affective Disorder (SAD) that extending the photoperiod in the morning and evening with bright full-spectrum light leads to a significant antidepressant response whereas equivalent treatment with dim light does not. We have shown that this antidepressant effect is not the result of sleep deprivation alone. However, since in the past there has always been some element of sleep deprivation involved in both active and control treatments, we could not rule out sleep deprivation as a contributing factor to the antidepressant response. Sleep deprivation occurred largely as a result of patients having to wake early in the morning for light treatment. This past year we explored the effects of light treatment given in the evening only without any element of sleep deprivation. This also enabled us to investigate whether the early morning hours are critical for the antidepressant response to light, as has been suggested to be the case for sleep deprivation. In nine patients we studied the antidepressant effects of five hours of bright (2500 lux) lux given after dusk and compared the results to an equivalent amount of dim (300 lux) light given at the same time. Although the bright light appeared to be somewhat effective, there was no significant difference in the degree of mood change when effects of bright and dim light were compared with each other.

Further studies will continue to explore the formal properties of the antidepressant effects of light in SAD. We are especially curious to determine whether, given equal amounts of bright light, the timing of the administration of the light will affect outcome.

Project Description:

Previous studies have shown that in the winter depressions of patients with Seasonal Affective Disorder (SAD), extending the photoperiod in the morning and evening with bright environmental light has an antidepressant effect. During the past year we explored whether extending the photoperiod with evening light alone would have similar antidepressant effects.

Methods

Patients were recruited via the media and community referrals. They were screened for a history of SAD and were followed clinically from summer into winter. When they became depressed [Hamilton Rating Scale (HRS) > 13], they were randomly assigned to one week of bright (2500 lux) or dim (300 lux) light for five hours after dusk. Patients were then withdrawn from light treatment for a week and then the alternate type of light treatment was administered. Mood was rated by blind raters after each week. Patients were advised to maintain their usual sleep schedules.

Findings to date:

Nine people completed the study. There was a significant difference between HRS scores before and after bright light treatment ($p < .05$). No such difference was observed when pre- and post-treatment values for dim light were compared. However, the difference between the effect of bright and dim light was not large enough to show a significant difference in HRS change scores for the two treatment conditions.

In all three light studies previously performed, where three hours of light treatment were administered both in the morning and the evening, we were able to show significant bright-dim differences in the HRS change scores. We conclude that the evening light treatment given in this study, though somewhat effective, was not as effective as the morning and evening light treatments administered in previous studies. The reasons for this (operating either individually or in combination) may be that (1) evening is a time when SAD patients are less sensitive to light than they are during the morning hours; (2) the amount of light administered in this study was less than that given in previous studies (five hours compared to six hours); and (3) an element of sleep deprivation, which was present in earlier studies and was absent in this study, contributes to the antidepressant effect of light treatment.

Significance to Biomedical Research and to the Program of the Institute:

1. The introduction of light as an antidepressant treatment promises relief to the large numbers of people who suffer from SAD, a condition for which light appears to be both an effective and a safe treatment. For many people it is inconvenient and tiring to wake in the early hours of the morning for light treatment. It appears that certain people might be able to benefit from light administered for five hours in the evening only. However, for those who are not helped in this way, morning

and evening light combined might still be effective as the latter treatment appears to be more effective.

2. It is of theoretical interest to define the times when individuals are sensitive to the antidepressant effects of light. When this is known, we will be closer to understanding the mechanism by which light exerts its effects.

3. Patients with SAD may represent the extreme in the spectrum of human vulnerability to seasonal changes. Many people who would not meet the criteria for SAD may well be influenced by climatic changes, resulting in human suffering and decreased productivity. Environmental light manipulations might improve the quality of life for such people.

4. Light treatment may be beneficial in conditions other than SAD. Non-seasonal depression and delayed phase sleep syndrome are two likely possibilities. Our demonstration of the therapeutic efficacy of light may encourage others to explore its use in different clinical settings.

Proposed Course:

We plan to continue exploring whether timing of light treatment is important in obtaining an antidepressant effect. We plan to undertake a skeleton photoperiod study; i.e. we plan to expose patients to two three-hour pulses of bright light in a light-insulated environment under two separate conditions. In the one case the bright light intervals will be relatively close together (separated by 3 hours of dim light); in the other case the pulses will be farther apart (separated by 6 hours of dim light). Animal studies suggest that the former condition is perceived as a short, 9-hour photoperiod and the latter as a long, 15-hour photoperiod. We will test whether the effects of these two light conditions differ. We hope to learn more about the mechanism of light's effect regardless of the experimental outcome. If there is a difference in the outcome, this would be a further indication that the antidepressant effects of light are mediated by a psychobiological mechanism rather than by psychological suggestion, and that the timing of light (i.e. a photoperiod mechanism) is involved.

Publications:

Rosenthal, N.E., Sack, D.A., Carpenter, C.J., Parry, B.L., Mendelson, W.B., Wehr, T.A. Antidepressant effects of light in seasonal affective disorder. Am J of Psychiatry (In Press).

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02206-02 CP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neurobiology of Seasonal Affective Disorder (SAD)

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: N. E. Rosenthal Chief, Outpatient Services CPB/NIMH

Others: D. A. Sack Chief, Inpatient Services CPB/NIMH
 S. P. James Clinical Associate CPB/NIMH
 B. L. Parry Clinical Associate CPB/NIMH
 W. B. Mendelson Chief, Unit on Sleep Studies CPB/NIMH
 T. A. Wehr Chief, Clinical Psychobiology Branch CPB/NIMH

COOPERATING UNITS (if any)

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INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

1.2

PROFESSIONAL:

0.4

OTHER:

0.8

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

There is a well-established epidemiological association between the incidence of affective episodes and suicides and the seasons. Our group has previously described patients who regularly become depressed each winter, and we have called this syndrome Seasonal Affective Disorder (SAD). One approach to understand how changes in mood in this population are altered by seasonal changes in the environment is to study physiological and biochemical parameters at different times of the year in people whose mood is vulnerable to seasonal changes, and in healthy controls. This may yield insights into the mechanism of seasonal influences on the incidence of affective episodes in the general population.

In eight patients we showed that during the winter, sleep length was significantly increased (a replication of an earlier finding), REM density was significantly increased and delta sleep tended to be decreased.

Several summer-winter studies on small numbers of patients showed no seasonal effect. These included basal metabolic rate (N=10), glucose tolerance tests (N=7), and urinary 6-OH-melatonin (N=6).

In only 2 out of 13 dexamethasone suppression tests (DSTs) performed on SAD patients during the winter was there a failure to suppress cortisol secretion normally. This concurs with our previous finding that in the winter depressions of SAD patients DSTs are generally normal.

In a preliminary study, hourly overnight plasma samples were drawn in four SAD patients and three normal controls in winter before and after a week of morning and evening light treatment. Patients showed higher baseline levels of melatonin and appeared less sensitive to the melatonin-suppressing effects of light than the volunteers.

We are planning to follow up this interesting preliminary finding with systematic circadian studies of melatonin and cortisol in summer and winter in a larger number of patients and normals.

Project Description:

There is a well-established epidemiological association between affective episodes, suicides and the seasons.

The introduction of light as an antidepressant treatment promises relief to the large numbers who suffer from Seasonal Affective Disorder (SAD), a condition for which light appears to be both effective and safe. The therapeutic effects of light may extend to other conditions, e.g., non-seasonal affective disorder.

We have attempted to understand how the changing seasons might alter mood by studying physiological variables at different times of the year.

Selection of Subjects:

SAD patients were screened and selected on the basis of the following criteria:

- (a) met RDC criteria for major affective disorder at some time in their lives;
- (b) depressions occurred during at least two successive winters, and remitted by the following summers;
- (c) there were no obvious seasonally fluctuating psychosocial causes to account for the seasonal occurrence of depressions.

Methods:

1. Nine subjects with SAD and eight normal controls had EEG recordings during sleep for one adaptation night and three subsequent nights both summer and winter.
2. During their admission 24-hour urine collections were obtained from patients. Samples were assayed for 6-hydroxy-melatonin by GCMS.
3. Basal metabolic rate studies were performed on 10 SAD patients during summer and winter.
4. Standard dexamethasone suppression tests (DSTs) were performed on 13 depressed SAD patients. One mg of dexamethasone was administered at 11:00 p.m. and the patient's blood was drawn and assayed for cortisol at 4:00 p.m. the next afternoon.
5. Overnight plasma samples were collected on an hourly basis from 4 SAD patients during the winter before and after one week of morning and evening light treatment.

Findings to date:

1. In the winter sleep length increased by 19% ($p < .02$) and REM

density increased by 43% ($p < .01$). The increased sleep length replicated our earlier finding in nine other SAD patients. Although delta sleep decreased in winter by 18%, this did not reach statistical significance as had occurred in our earlier study.

2. In six patients where we measured 6-OH-melatonin summer and winter, we found no seasonal differences.

3. Glucose tolerance tests performed in 7 patients summer and winter revealed no seasonal differences.

4. Similarly no summer-winter differences were noted in basal metabolic rate in 10 patients.

5. Dexamethasone suppression tests in 13 depressed SAD patients revealed failure to suppress cortisol response in only two cases. If one considers that seven similar tests performed in a previous study were also all negative, the incidence of DST non-suppressors among depressed patients with SAD appears to be rather low (10%), no higher than the incidence in the general population.

6. In four depressed SAD patients hourly plasma melatonin levels between 6:00 p.m. and 2:00 a.m. appeared higher than in three control subjects. Patients with SAD also appeared less sensitive to the melatonin-suppressing effects of light than did normal volunteers. The numbers are too small at present for meaningful statistical examination.

Significance to Biomedical Research and to the Program of the Institute:

1. A large number of people appear to be adversely affected by changes in seasons and the weather. It would be valuable to learn how these changes are mediated. This might contribute to our ability to protect people from these adverse effects.

2. Since seasonal variation appears to be a cardinal feature of affective disorders, an understanding of the pathophysiology of these changes might expand our understanding of the mechanisms underlying affective disorders.

3. The confirmation of abnormal sleep architecture in patients with SAD during the winter months constitutes the first replicated pathophysiological marker of this condition.

Proposed Course:

We plan to study the circadian rhythms of melatonin, cortisol, prolactin and growth hormone at different times of the year. All these hormones have been implicated in the changes seen in depression, with the changing seasons or in both situations.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02221-01 CP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (90 characters or less. Title must fit on one line between the borders.)

Effects of Melatonin in Seasonal Affective Disorder (SAD)

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	N. E. Rosenthal	Chief, Outpatient Services	CPB/NIMH
Others:	D. A. Sack	Chief, Inpatient Services	CPB/NIMH
	S. P. James	Clinical Associate	CPB/NIMH
	B. L. Parry	Clinical Associate	CPB/NIMH
	T. A. Wehr	Chief, Clinical Psychobiology Branch	CPB/NIMH
	W. B. Mendelson	Chief, Unit on Sleep Studies	CPB/NIMH
	L. Tamarkin	Research Biologist	CPB/NIMH

COOPERATING UNITS (if any)

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NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

1.2

PROFESSIONAL:

0.4

OTHER:

0.8

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Seasonal Affective Disorder (SAD) is a condition which affects people on an annual basis and is characterized by retarded depression, overeating, oversleeping and carbohydrate craving in the winter and euthymia or hypomania in the spring and summer. It resembles the annual rhythms of reproduction, which are widespread among other animals, and like these rhythms can be modified by extending the winter photoperiod. We have shown that three hours of bright environmental light before dawn and after dusk frequently reverses the symptoms of SAD. In other species the pineal hormone, melatonin, has been shown to mediate the effects of photoperiod. In certain studies the duration of the melatonin pulse, which is inversely related to the duration of the photoperiod, has been shown to be the critical aspect of melatonin secretion, which influences photoperiodic responses. In hamsters and sheep, for example, melatonin administered orally in the evening during the long summer days, has been shown to elicit short-day, winter responses. We have explored whether melatonin, administered orally in the evening and morning hours, will reverse the effects of extending the photoperiod with bright light. In eight SAD patients, melatonin and placebo were given for one week each after patients had been successfully treated with bright light. Melatonin did not fully reinduce the typical winter depression but did induce some of its classical symptoms such as overeating, fatigue and oversleeping. Further studies will explore whether patients can be helped by blocking melatonin secretion.

Project Description:

Patients with well-documented SAD were treated with bright environmental light for three hours before dawn and three hours after dusk. Following an antidepressant response melatonin was given orally at times corresponding to the times of light treatment. We hypothesized that if melatonin was important in mediating the symptoms of SAD, these symptoms should return following oral melatonin administration.

Methods:

Patients were recruited and screened in the summer or fall of 1983. They were followed longitudinally as they became depressed and their mood was monitored by means of the 23-item Hamilton Rating Scale (HRS). When they became depressed (HRS > 13) they were treated with bright (2500 lux) full-spectrum fluorescent light for three hours before dawn and three hours after dusk. Once they had responded, they were given approximately 2 mg of melatonin per day in divided doses at the times of light treatment, over a period of a week.

Placebo was similarly administered for a week. The order of treatments was randomized and both patients and raters were blind to the type of treatment given.

Wherever possible overnight plasma melatonin levels were drawn each hour before light treatment, after a week of melatonin and after placebo.

Findings to date:

1. Patients reported a significant mood improvement following bright light treatment.
2. The administration of melatonin did not reproduce the typical symptoms of depression as rated by the 23-item HRS. A cluster of depressive items typical of SAD but not included in the HRS were reproduced by melatonin to a significantly greater degree than by placebo ($p < .001$). These symptoms include overeating, oversleeping, weight gain, carbohydrate craving, fatigue and social withdrawal.
3. In the four subjects from whom we were able to obtain melatonin levels, we found that oral melatonin administration more than compensated for the suppressing effects of light, creating higher than normal melatonin levels throughout the night.

Significance to Biomedical Research and to the Program of the Institute:

1. We cannot say, based on our data, to what extent melatonin may account for the symptoms of SAD. However, it does reproduce a significant cluster of symptoms. It may perhaps be responsible for the development of these symptoms in vulnerable individuals, and for their response to light. It is possible that some artifact of the way in which melatonin was administered e.g. the timing or the duration of administration (only one week) may account for why melatonin did not reinduce all the symptoms of SAD.

2. If melatonin is involved in the pathogenesis of SAD, we may be able to devise more effective ways of treating the condition by blocking melatonin secretion.

3. The evaluation of melatonin's role in SAD may assist us in understanding how normal people respond to changes in the photoperiod.

4. The effects of light and melatonin on circadian and annual rhythms and on behavior are of interest to basic researchers. To study these effects in humans is a logical extension of their basic work and will expand our knowledge of circadian and circannual physiology.

Proposed Course:

We plan to study in humans the effects of administering medications which have been shown to block melatonin production in animals. In animals melatonin can be blocked by beta-adrenergic blockers such as propranolol and by alpha adrenergic blockers such as prazosin. These drugs have been used with safety in humans and may be of use both in treating SAD and in further clarifying the role of melatonin in this condition.

Rosenthal, N.E., Sack, D.A., Wehr, T.A. Seasonal effects on mood: The role of light. Encyclopedia of Neuroscience, G. Adelman (ed), Birkhauser Boston, Inc. (In Press).

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02222-01 CP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Treatment of Rapid-Cycling Manic-Depressive with Thyroxine Illness

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: D. A. Sack Chief, Inpatient Services CPB/NIMH

Others: T. A. Wehr Chief, Clinical Psychobiology Branch CPB/NIMH
 N. E. Rosenthal Chief, Outpatient Services CPB/NIMH
 B. L. Parry Clinical Associate CPB/NIMH
 W. B. Mendelson Chief, Unit on Sleep Studies CPB/NIMH
 S. P. James Clinical Associate CPB/NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Psychobiology Branch

SECTION

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS

3

PROFESSIONAL:

2

OTHER:

1

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Previous studies have shown that patients with rapid-cycling manic depressive illness are more likely to develop lithium induced hypothyroidism and goiter than patients with less frequent relapses. These patients also develop elevated thyroid stimulating hormone levels (TSH) in the presence of normal thyroid indices when they are treated with lithium. Previous open clinical trials suggest that the mood disorder of rapid cycling patients may improve with hypermetabolic doses of thyroxine but as yet controlled clinical trials are lacking. Previous controlled trials of thyroxine were not effective in non-rapid cycling depressed patients suggesting that the abnormalities in thyroid function and a clinical response to thyroxine are defining characteristics of the rapid-cycling bipolar population.

As part of a continuing study, we are further delineating the nature and etiology of the hypothalamic-pituitary thyroid dysregulation in rapid cycling patients. Studies include circadian profiles of TSH drawn every 30 minutes, morning and evening TRH stimulated TSH responses, and determination of the pituitary set point to thyroxine. Together these studies should enable us to determine whether the abnormalities of thyroid regulation seen in these patients arise from changes at the level of the hypothalamus, the pituitary gland or the thyroid gland.

Following these evaluations patients undergo a double-blind controlled trial of euthyroid and hypermetabolic dose of thyroxine for the treatment of their affective disorder. In addition to clinical measures, this study will enable us to assess the important interactions between thyroxines, norepinephrine, and mood, as measured by changes in the whole body norepinephrine turnover during the course of treatment.

Project Description:

The regulation of thyroid hormones through the hypothalamus and pituitary gland appears abnormal in rapid-cycling, manic-depressive patients. These patients exhibit a greater tendency to develop lithium-induced hypothyroidism, goiter and they develop elevated TSH levels when treated with lithium despite normal circulating T₃ and T₄ levels. These findings suggest that rapid-cyclers are more prone to develop hypothyroidism than patients with other affective disorders and that their pituitary glands may be relatively insensitive to the effects of circulating thyroid hormone.

If rapid cycling patients manifest a deficiency of thyroid hormones and/or a relative insensitivity to circulating thyroid hormones, as these findings suggest, then the clinical course of these patients should improve when they are treated with hypermetabolic doses but possibly not euthyroid doses of thyroxine.

The objectives of this study are:

1. To determine the specific abnormalities of H.P.T. regulation which characterize bipolar, rapid-cycling (B.P.-R.C.) patients.
2. To determine whether bipolar, rapid-cycling patients are physiologically less sensitive to thyroxine.
3. To determine the relative efficacy of euthyroid and hypermetabolic doses of thyroxine in the treatment of the mood disorders of B.P.-R.C. patients.

Methods:

Patients are included if they meet RDC criteria for RPI or BPII disorder and are free of psychotropic medications for at least three weeks. Rapid-cycling is defined as a history of at least four or more episodes of mania or depression in a one year period at some point in their course of illness.

The H.P.T axis is assessed by the following studies:

1. Baseline T₃ RIA, T₄, Free T₄, TSH.
2. Circadian blood study for 48 hours with samples drawn every 30 minutes for TSH, with sleep deprivation on the second night.
3. TRH stimulated TSH test performed at 7 a.m. and at 11 p.m. at least one week apart.

Following these studies the TSH set point is determined. This set point is the dose of thyroxine required to suppress the pituitary response to a standardized infusion of TRH. It is a relative measure of cellular sensitivity to the effects of thyroid hormone in the pituitary gland.

Once the set point has been determined all patients will undergo a clinical trial of thyroxine. Initially all patients will be treated

with a suppressive (euthyroid) dose of thyroxine. Those whose mood fails to improve are then randomized to hypermetabolic treatment or continued suppressive therapy. This design ensures that no patient will be made clinically hyperthyroid as part of the trial unless they fail to improve with suppressive therapy.

Thus far four patients have been studied, but only two have completed the trial of thyroxine. Of these, one patient improved dramatically on hypermetabolic doses of thyroxine but not on suppressive doses. The other failed to improve with either regimen. Interpretation of the H.P.T. evaluations will require additional subjects and normal controls.

Significance to Biomedical Research and to the Program of the Institute:

1. Approximately 15% of all manic-depressives respond poorly to lithium carbonate and of these the majority are rapid-cyclers. These patients constitute a large refractory group for whom present treatments are inadequate.
2. Rapid cyclers demonstrate a higher incidence of thyroid abnormalities than other bipolar patients. The anecdotal literature suggests that rapid cyclers may respond to treatment with thyroxine whereas non-rapid cycling bipolars do not improve with thyroxine alone. Thus, these patients provide a model in which to study physiological interactions between thyroxine and neurotransmitters on mood.

Proposed Course:

Over the next year we intend to study six additional rapid cycling patients. We will also obtain normative data on the circadian rhythm for TSH as well as the pituitary set point on age and sex matched controls.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02223-01 CP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pentobarbital and Ethanol Toxicity: Relation to the Benzodiazepine Receptor

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: W. B. Mendelson Chief, Unit on Sleep Studies CPB/NIMH

Others: J. V. Martin Staff Fellow CPB/NIMH
 C. Roseberry Biological Laboratory Technician CPB/NIMH
 R. Wagner Guest Worker CPB/NIMH

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NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

1.5

PROFESSIONAL:

0.3

OTHER:

1.2

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Both barbiturates and ethanol have been reported to interact with the GABA-benzodiazepine receptor-chloride ionophore "supramolecular complex". These observations raise the possibility that some of the pharmacologic actions of barbiturates and ethanol may be mediated through this complex. In this study we have administered a series of drugs which bind to various components of the complex in an attempt to antagonize the lethality of sodium pentobarbital and loss of righting reflex induced by ethanol in mice. It was found that isopropylbi-cyclophosphate (IPPO), a cage convulsant which binds at or near the chloride ionophore, greatly reduces the overall mortality (and increases latency to death) of animals pretreated with a lethal dose of pentobarbital. Picrotoxin also decreases pentobarbital lethality, but only at doses which were usually lethal when given alone. Picrotoxin shortened, rather than increased, latency to death. Strychnine did not prevent pentobarbital lethality, suggesting that the IPPO effect is not shared by convulsants in general. IPPO did not prevent ketamine-induced deaths, which supports the notion that the protective actions of IPPO are specific for depressant drugs which act at the chloride ionophore. IPPO also significantly reduced the duration of loss of righting reflex induced by ethanol. These observations suggest that the use of compounds which have a high affinity for the chloride ionophore in vitro might be fruitful in developing a clinical treatment for barbiturate or ethanol toxicity.

Project Description:

Barbiturates such as pentobarbital have been shown to interact with the GABA-benzodiazepine receptor-chloride ionophore "supramolecular complex", apparently at a site on or near the chloride ionophore. The neurochemical consequences of this interaction include an increase in the apparent affinity of [³H] benzodiazepines (e.g. flunitrazepam, diazepam) and increases in both the number of GABA receptors and the apparent affinity of [³H] GABA and GABA mimetics. The good correlation obtained between the alterations in benzodiazepine receptor affinity and the potency of a series of barbiturates as anaesthetics suggests that perturbation of this supramolecular complex by barbiturates may be responsible for the electrophysiological and pharmacological properties shared by both benzodiazepines and barbiturates.

Ethanol has also been reported to increase the apparent affinity of benzodiazepines in both membrane and solubilized preparations. Since ethanol shares common pharmacologic properties with both barbiturates and benzodiazepines (e.g. anxiolytic, muscle relaxant, sedative/hypnotic properties), it is possible that this supramolecular complex may be responsible for mediating the common pharmacologic properties of these chemically diverse compounds.

If this hypothesis is correct, then compounds which bind at the chloride ionophore or some other component of the complex might block some or all of the pharmacologic actions of barbiturates and ethanol. Neurochemical evidence supports this hypothesis, since bicuculline, which binds to GABA receptors, can reverse barbiturate-induced increases in the affinity of [³H] benzodiazepine binding. Furthermore, picrotoxin, which binds to chloride ionophore sites with a moderate affinity has been used clinically to treat barbiturate poisoning, although its usefulness is limited due to its toxicity. Finally the benzodiazepine antagonist CGS 8216 has been shown to reverse the anticonflict actions of pentobarbital, and picrotoxin has been reported to reverse the anticonflict actions of oxazepam. Thus, we initiated pharmacologic studies to determine if compounds which interact with different components of the supramolecular complex could be used to reverse pentobarbital (PB) and ethanol toxicity.

We now report that isopropylbicyclopophosphate (IPPO), a "cage convulsant" which binds at or near the chloride ionophore, significantly reduces the lethal actions of pentobarbital and reduces the duration of ethanol-induced loss of the righting reflex (LRR). These actions exhibit some degree of specificity, since IPPO did not influence the toxicity of ketamine, an anaesthetic which does not appear to interact with the supramolecular complex. Strychnine, a convulsant which presumably acts as a glycine antagonist, failed to reverse the lethal actions of pentobarbital. Picrotoxin was also found to antagonize pentobarbital-induced lethality, but only at doses which produce a significant number of deaths when given alone. These findings suggest that it may be possible to design molecular antagonists of the pharmacologic actions of barbiturates and ethanol based on their interactions at the GABA-benzodiazepine receptor-chloride ionophore complex.

Methods:

Rats were treated with pentobarbital or ethanol followed by a variety of agents which lead to various components of the BZ receptor complex, as seen in the attached table.

Findings to Date:

As described in the summary, IPPO greatly reduced mortality from high doses of pentobarbital, and reduced duration of loss of righting reflex due to ethanol.

Significance to Biomedical Research and to the Program of the Institute:

The perturbation of the GABA-benzodiazepine receptor-chloride ionophore complex by PB and ethanol in vitro suggests that some of the pharmacologic properties of these agents could be mediated at these sites. These in vitro studies are consistent with in vivo evidence that picrotoxin and CGS 8216 may reverse some actions of PB. This led to the present study in which the effects of a series of ligands binding to various components of the supramolecular complex were examined for their abilities to reverse PB and ethanol toxicities. It was found that IPPO, which binds to the chloride ionophore, can partially reverse the toxic effects of pentobarbital in a dose-dependent manner. Picrotoxin, which presumably binds at the same site and has been used clinically in barbiturate poisoning, can also partially prevent pentobarbital lethality. However, at equieffective doses, picrotoxin is significantly more toxic than IPPO and, in contrast to IPPO, shortens the latency to death. Two other agents that bind to the dihydropicrotoxinin site, MT 11 and cartazolol, had no effect on PB mortality. These findings suggest that binding at or near the chloride ionophore per se is not sufficient, and that protective effects are dependent on a more subtle interaction of ligand and receptor. Strychnine does not affect pentobarbital-induced mortality, suggesting that the effects of IPPO are specific and not common to all convulsants. Further, IPPO did not prevent ketamine mortality, suggesting that its effects may be restricted to depressants which are ligands at the chloride ionophore site (or supramolecular complex). A dose-dependent effect on ethanol-induced LRR was also seen with IPPO. These observations suggest that some aspects of the toxic effects of pentobarbital and ethanol may be reversed by ligands which bind to the supramolecular complex.

Although the toxicity of IPPO is substantially less than that of picrotoxin, its clinical usefulness appears limited. However, our results clearly show that various ligands which bind to the dihydropicrotoxinin site can have widely varying potencies in protection from pentobarbital mortality. Further, the potencies are not necessarily correlated with the toxic effects of the ligands given alone. These observations suggest that it would be fruitful to use this approach to find a related compound for the treatment of toxicity from barbiturates or ethanol.

Proposed Course:

We are continuing to evaluate a series of compounds which act at the receptor complex in order to find one which is even more effective and more benign when given alone.

Summary of Experimental Design and Results

Injection 1 ^{1,2}	Injection 2	% Seizures with Vehicle as Injection 1	% Deaths with Vehicle as Injection 1	% Deaths with Active Drugs as Injection 1
PB or Vehicle	125-500 mg/kg IPPO or vehicle	8-80%	0-45%	58-87%
PB or Vehicle	4-16 mg/kg picrotoxin or vehicle	20-92%	0-90%	58-95%
PB or Vehicle	1.5-6 mg/kg strychnine or vehicle	30-75%	20-50%	100%
PB or Vehicle	7.5-30 mg/kg R5135 or vehicle	20-85%	0-90%	95-100%
PB or Vehicle	40-240 mg/kg MT II or vehicle	0-60%	0-15%	92-100%
PB or Vehicle	20-80 mg/kg Cartazolate or vehicle	0-55%	0-5%	80-90%
PB or Vehicle	5-20 mg/kg CGS 8216 or vehicle	0	0	80-98%
PB or Vehicle	30-60 mg/kg @GCT or vehicle	0	0	75-95%
Ketamine or Vehicle	125-500 mg/kg IPPO or vehicle	0-95%	0-55%	80-100%

¹Injection 1 was followed 5 min. later by Injection 2. Death latencies are measured from time of injection 1.

²The dose of pentobarbital was always 170 mg/kg. The dose of ketamine was 300 mg/kg.

³In those animals who received vehicle for both injections, there were no deaths.

⁴In each experiment, a group of mice were given active drug as injection 1, followed by the vehicle for the drug given at the second injection. Pentobarbital caused an average of 94 + 4% deaths (range=75-100%). The dose of ketamine used caused 80% of the mice to die.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02224-01 CP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Effects of Dihydropyridines on Benzodiazepine-Induced Alterations in Ca^{2+} flux

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: W. B. Mendelson Chief, Unit on Sleep Studies CPB/NIMH

Others: J. V. Martin Staff Fellow CPB/NIMH

COOPERATING UNITS (if any)

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TOTAL MAN-YEARS:

1.5

PROFESSIONAL:

0.3

OTHER:

1.2

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Pharmacologically relevant concentrations of benzodiazepines have previously been reported to increase $^{45}Ca^{2+}$ uptake into synaptosomes. This observation, coupled with the recent report that nifedipine may block the hypnotic effect of flurazepam, led us to study the effects of nifedipine and nitrendipine on $^{45}Ca^{2+}$ uptake into synaptosomes. Both agents had little effect by themselves, but prevented enhancement of uptake by diazepam. These observations further support the notion that benzodiazepine receptors in brain may be coupled to a calcium channel which may have some relevance to the actions of benzodiazepines.

Project Description:

Benzodiazepines have well-described postsynaptic actions to enhance the inhibitory events mediated by GABA. In addition, benzodiazepines have also been reported to affect presynaptic events such as GABA-mediated presynaptic inhibition in spinal cord and the depolarization-induced release of GABA from rat cortical slices. Recently, we described a benzodiazepine-stimulated stereoselective increase in $^{45}\text{Ca}^{2+}$ uptake into brain synaptosomes which occurs under depolarizing conditions. This action was observed at pharmacologically relevant concentrations of benzodiazepines and was blocked by CGS 8216, a specific benzodiazepine receptor antagonist. These observations suggest that benzodiazepine-stimulated changes in synaptosomal calcium flux are mediated by specific receptors for benzodiazepines. The possible role of calcium in at least some of the pharmacologic actions of the benzodiazepines is supported by the recent observation that intraventricular administration to rats of the dihydropyridine calcium channel antagonist nifedipine will block sleep induction by flurazepam at doses which do not affect sleep when given alone. Putative calcium channels in brain have been identified using ^3H nitrendipine, a potent calcium channel antagonist. The characteristics of these calcium channels in the central nervous system resemble those found in peripheral tissues. Hirsch and Kochman have suggested that these putative calcium channels are tightly coupled to the benzodiazepine receptor complex, thus supporting our in vivo and in vitro observations of the possible role of calcium in some of the therapeutic actions of the benzodiazepines.

We now report that the dihydropyridine calcium channel antagonists nifedipine and nitrendipine can antagonize diazepam-stimulated increases in $^{45}\text{Ca}^{2+}$ uptake into potassium-depolarized synaptosomes. This further supports the notion that benzodiazepine receptors in brain may be coupled to a calcium channel and that alterations in calcium flux may be responsible for some of the pharmacologic actions of the benzodiazepines.

Methods:

Nifedipine and nitrendipine were added to incubation mixtures of synaptosomal preparations with diazepam and labeled calcium.

Findings to Date:

The effects of nifedipine and nitrendipine, two closely related dihydropyridine analogs, were examined in separate series of experiments. As previously reported diazepam (1 μM) significantly increased $^{45}\text{Ca}^{2+}$ uptake into potassium-depolarized synaptosomes. Despite variation in the basal (i.e., no drug) uptake of calcium into synaptosomes, diazepam was consistent in stimulating $^{45}\text{Ca}^{2+}$ uptake, 75 and 78% above control values. Addition of either nifedipine or nitrendipine (1 μM) completely reversed the increased $^{45}\text{Ca}^{2+}$ uptake by diazepam. Nitrendipine, but not nifedipine, appeared to

significantly reduce basal uptake of calcium.

Significance to Biomedical Research and to the Program of the Institute:

Several lines of evidence have recently been provided which link benzodiazepine receptors to calcium flux in neurons. The demonstration of calcium channels in brain with the dihydropyridine calcium channel antagonists and the subsequent demonstration that these agents reverse a pharmacologic action of a benzodiazepine prompted a study of the effects of calcium channel blockers on diazepam-stimulated increases in calcium flux. The blockade of diazepam-stimulated increases in calcium uptake by nifedipine and nifedipine further supports the role of calcium in mediating at least some of the pharmacologic actions of the benzodiazepines.

Proposed Course:

We hope to examine the role of non-dihydropyridine calcium channel blockers in this model.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02225-01 CP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies in the Role of Calcium Flux in the Sleep-Inducing Effects of Flurazepam

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: W. B. Mendelson Chief, Unit on Sleep Studies CPB/NIMH

Others: J. V. Martin Staff Fellow CPB/NIMH

R. Wagner Guest Worker CPB/NIMH

COOPERATING UNITS (if any)

LBC/NIADDK
NSB/NIMH

LAB/BRANCH

Clinical Psychobiology Branch

SECTION

Unit on Sleep Studies

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

1.5

PROFESSIONAL:

0.3

OTHER:

1.2

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Previous studies have implicated the benzodiazepine receptor in the sleep-inducing effects of these widely-used hypnotics, but the effector mechanism of this process is poorly understood. There is also *in vitro* evidence that benzodiazepines enhance calcium entry into synaptosomal preparations, leaving open the possibility that altered calcium flux may be involved in their actions. In order to explore this hypothesis, we administered intraventricular nifedipine, a calcium blocking agent. It was found that pretreatment with a dose of nifedipine which by itself does not affect sleep will prevent sleep-induction by flurazepam in rats. Effects on anticonvulsant properties of flurazepam were not apparent. This seems to suggest that changes in calcium channel function may be involved in the hypnotic action of benzodiazepines.

Project Description:

It is now well established that the anxiolytic, anticonvulsant and muscle relaxant properties of the benzodiazepines are mediated by interaction with high-affinity, stereospecific receptors. Recent work from this laboratory suggests that this is also true for the sleep-inducing properties, insofar as low doses of 3-hydroxymethyl-B-carboline, which binds to these receptors, prevents sleep-induction by flurazepam. The mechanism by which receptor stimulation leads to pharmacologic effect has not been established. Although it is generally well accepted that benzodiazepines potentiate GABA-mediated chloride conductance, other ionic effects have also been proposed. There have been reports that benzodiazepines enhance calcium entry into synaptosomal preparations, an observation compatible with the hypothesis that altered calcium flux may be involved in the actions of benzodiazepines. Recent studies from our laboratories have shown that pharmacologically relevant concentrations of benzodiazepines selectively enhance the potassium-depolarized uptake of calcium into cerebral cortical synaptosomes. These effects are blocked by the benzodiazepine antagonist CGS 8216 and the GABA antagonist bicuculline. Administration of calcitonin (which lowers serum calcium concentrations) has been reported to decrease sleep in humans. These observations led us to examine the interaction between nifedipine, a calcium channel blocker, and flurazepam on sleep in the rat.

Methods:

Animals were administered vehicle or 40 ug/kg nifedipine intravenously, and were then given vehicle or 40 mg/kg flurazepam. Standard two-hour sleep recordings were then performed.

Findings to Date:

Under these experimental conditions, intraventricular injection of nifedipine had no effect on any of the sleep parameters examined. In contrast, flurazepam had a potent hypnotic effect, reducing sleep latency from 26.5 ± 5.1 min to 12.6 ± 1.3 min ($p < 0.01$) and enhancing non-REM sleep ($p < 0.02$). In confirmation of previous studies, there were no major effects on REM sleep. Pretreatment with nifedipine blocked the hypnotic effects of flurazepam. Sleep latency in the "nifedipine plus flurazepam" group rose (25.6 ± 5.0 min) to levels indistinguishable from the vehicle controls. Similarly, total sleep and non-REM sleep in this group were similar to control values.

Significance to Biomedical Research and to the Program of the Institute:

It has been previously reported that benzodiazepines enhance calcium uptake into synaptosomes, and this effect is blocked by the benzodiazepine receptor antagonist CGS 8216. These observations are consistent with the

hypothesis that calcium ion flux is part of the effector mechanism subsequent to occupation of benzodiazepine receptors. This hypothesis is further supported by the recent finding that nifedipine prevents the diazepam-induced increase in synaptosomal calcium uptake. The present data may further demonstrate a specificity in function of the calcium-mediated aspect of benzodiazepine action. Calcium ions may be more directly involved in sleep regulation than in other effects of benzodiazepines.

The exact site of action of calcium channel blockers such as nifedipine in the brain is not yet established. Further study of the relationship of benzodiazepines and calcium flux may aid in elucidating the mechanism of action of these widely used agents.

Proposed Course:

1) We are now examining the effects of nifedipine on other properties of benzodiazepines.

2) We hope to examine the possible role of calcium flux in actions of non-benzodiazepine hypnotics.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02226-01 CP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Relation of Rhythms of Core Temperature and Sleep

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: W. B. Mendelson Chief, Unit on Sleep Studies CPB/NIMH

Others: N. Rosenthal Chief, Outpatient Services CPB/NIMH

D. Sack Chief, Inpatient Services CPB/NIMH

T. Wehr Chief, Clinical Psychobiology Branch CPB/NIMH

COOPERATING UNITS (if any)

BEIB/NIH

LAB/BRANCH

Clinical Psychobiology Branch

SECTION

Unit on Sleep Studies

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:

1.5

PROFESSIONAL:

0.3

OTHER:

1.2

CHECK APPROPRIATE BOX(ES)

 (a) Human subjects (b) Human tissues (c) Neither (a1) Minors (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The circadian rhythms of core temperature and the sleep/waking cycle are both well-described, but to date their interrelationship has been studied primarily by two means: observations under free running conditions, and after manipulations of the sleep schedule. The present work represents a novel approach: observation of sleep and waking when the core temperature rhythm is held constant. This is accomplished by a "space suit" and computer-driven cooling and heating system developed by the investigators. Using this apparatus, the rhythm of core temperature is abolished, and measures of sleep and subjective experience and motor performance are measured.

Project Description:

Normal volunteers spend an adaptation night and one night of baseline sleep study in the laboratory. They then spend 24 hours on the ward while their core temperature is monitored, and subjective evaluation of wakefulness mood, and cognitive and motor performance measures are taken. A week later they spend a second 24 hour period during which their temperature is held at the mean value from the previous study, and the various measures are taken.

Methods:

Sleep studies are performed by standard techniques. The temperature of the subjects is measured by a rectal probe and also by a sensor on the chest wall. This data goes to a computer, which then directs warm or cool water to flow through the lining of the "space suit".

Findings to Date:

Currently seven subjects have been successfully run in this protocol, and data is currently under analysis. A very preliminary suggestion is that sleep is relatively unaffected by abolishing the rhythm of temperature. A final assessment must await performance of the study on many more subjects.

Significance to Biomedical Research and to the Program of the Institute:

It is becoming more apparent that the numerous circadian processes which can be measured in humans may play a critical role in psychiatric illness, particularly affective disorders. Of particular interest is the hypothesis that in depressive illness there may be a phase advance of the REM sleep and core temperature rhythm relative to the sleep/waking rhythm. Thus data regarding the relationship of the two has a direct relevance to the study of affective disorders.

Proposed Course:

After the completion of the normal volunteer study, one might examine a possible treatment of depression by using this apparatus to realign the rhythm of temperature relative to the sleep waking cycle

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02227-01 CP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Insomnia: daytime and nighttime functioning

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: W. B. Mendelson Chief, Unit on Sleep Studies CPB/NIMH

Others: D. Garnett Psychology Technician CPB/NIMH

COOPERATING UNITS (if any)

LPP/NIMH

LAB/BRANCH

Clinical Psychobiology Branch

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INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

1.5

PROFESSIONAL:

0.3

OTHER:

1.2

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Ten insomniacs and controls, in whom major physiologic disorders such as sleep apnea and nocturnal myoclonus were ruled out, underwent studies of sleep, temperature, motor activity, cognitive performance, and perception of depth of sleep. Subjective descriptions of sleep differed significantly between insomniacs and normals on a variety of variables. In contrast, polysomnographic evaluation showed increased intermittent waking time and decreased sleep efficiency, and only a tendency toward decreased total sleep and increased sleep latency. Core temperature during sleep tended to be slightly higher than in controls, with no evidence of phase shift. MMPI evaluation revealed that insomniacs had higher scores on the F, D, and SI scales, and lower values on the K scale. On cognitive testing, insomniacs did well on tests of episodic (recent) memory, but displayed major deficits in accessing semantic memory (retrieval of material already known). Compared to normals, insomniacs described REM sleep as relatively "light" sleep.

Project Description:

The complaint of chronic poor sleep is, of course, very common, occurring in perhaps up to ten percent of the population. The major thrust of sleep research in recent years has been the discovery that insomnia is a complaint, often resulting from well-described pathophysiological processes such as sleep apnea, nocturnal myoclonus or circadian dysrhythmias. It is clear, however, that a large group of insomniacs remain in whom major pathophysiological processes have not been identified, and in whom certain psychological styles related to sleep have been found. In terms of the Association of Sleep Disorders Centers Nosology, these individuals appear as persistent psychophysiological, subjective DIMS complaint without objective findings, and not otherwise specified complaints. In these groups, relatively little is known about daytime consequences of (or concomitants to) a complaint of chronic poor sleep. There is little data available, for instance on whether the discomfort of the insomniac at night is translated into impaired performance by day. This is a critical question in assessing the utility of pharmacologic therapy, insofar as many hypnotics may affect daytime function. Similarly, although there have been several studies of MMPI scores in insomniacs and a few studies of personality qualities in insomniacs, this area is also still greatly in need of clarification. Finally, some classic studies of "poor sleepers" described alterations in temperature at sleep onset, and changes in perception of being asleep, but relatively little work has been done in populations which are now more well-defined. The present study was designed to provide a comprehensive view of ten insomniacs and matched controls in order to examine these questions. The goal was to characterize a number of aspects of these individuals, including: polygraphically and subjectively described sleep, daytime sleepiness, motor and cognitive performance, motor activity and temperature during sleep, personality measures, and perception of the experience of sleep.

Methods:

Ten chronic insomniacs and age-matched controls underwent a series of studies of sleep, rhythms of temperature and motor activity, and daytime performance.

Findings to Date:

In summary, carefully screened insomniacs were found to have mildly disturbed sleep, characterized primarily by increased waking time and decreased sleep efficiency by EEG criteria. There was also a tendency toward increased motor activity in the first two hours after EEG-defined sleep onset. In contrast, their description of habitual sleep patterns differed markedly from controls. MMPI data showed relatively little pathology compared to statistical norms, but did have significant differences compared to the control group. This suggested that insomniacs experience an enhanced sense of distress, depression and social introversion. Perhaps the most striking quality

of the insomniacs' responses to the various questionnaires was the contrast between the way they picture their habitual patterns and the way in which they described themselves on a moment-to-moment basis. The insomniacs describe themselves as habitually more tired during the day, and being less refreshed on awakening. When asked how they feel at a given moment in the daytime, however, both the 100 mm scales and the Mood Rating Scales showed no differences in being tired, sleepy, or degree of activation. At bedtime the insomniacs actually described themselves as less fatigued. A pegboard test and finger tapping test did not show major changes in daytime performance. On the other hand, cognitive testing indicated that, although insomniacs did well on episodic memory, they had clear impairment of semantic memory (the ability to retrieve and use material already well known). Core temperature data indicated that the insomniacs had a tendency toward an in-phase elevation of temperature which, at least at sleep onset and the first two hours of sleep, could not be explained by increased wakefulness. Arousal studies indicated that, compared to normals, insomniacs are more likely to identify REM sleep as "light" sleep.

Significance to Biomedical Research and to the Program of the Institute:

The polygraphic sleep data described here--which showed decreased sleep efficiency, an increase in intermittent waking, and a tendency toward less total sleep--is consistent with previous studies. The possibility should be borne in mind that with a larger number of subjects, more subtle differences in sleep (or other measures) between the two groups might emerge.

When correlations were performed between polygraphically--defined sleep parameters and daytime mood and performance, several qualities of the data became apparent. The first was the relative lack of relationship between the two. Particularly striking was the absence of relation of total sleep and sleep latency, measures commonly thought to be important in the cause of (and measures of treatment for) insomnia and its presumed daytime consequences. There was some evidence that intermittent waking time may be more related to daytime mood and performance, suggesting that it is the continuity, rather than total amount of sleep, which is important.

This study found that although insomniacs as a group did not differ significantly from normative data on the MMPI, significant contrasts with matched controls were found. A number of previous studies have also examined personality traits in insomniacs. In studies from Los Angeles and Hershey, 80-85 percent of insomniacs had an elevated score on at least one scale compared to 60 percent in the current study. Most earlier studies tended to find highest scores on the depression, hysteria, and psychasthenia scales. The present study found insomniacs rating higher on the depression scale. Thus, both clinical impression and formal testing continue to indicate that insomniacs differ in some personality qualities, which are still in need of more adequate description.

An objective measure of daytime sleepiness (Multiple Sleep Latency Test)

indicated no general tendency toward sleepiness in insomniacs. Values were approximately the same as controls, and only one patient out of ten could be said to have any evidence of sleepiness. This seems to confirm the MSLT study of Dement et al. in which the single most common value for insomniacs was 20 minutes. This suggests that whatever daytime distress is experienced by insomniacs is not manifest as objective sleepiness.

Cognitive testing results showed a very clear deficit in a specific aspect of memory processes, semantic memory, or the ability to retrieve well learned information that is part of one's knowledge base. Thus, although these patients can easily learn new information, they are less able to use what they already know. In addition, they may demonstrate impairment in manipulating and organizing information. Subjectively, this may be experienced as an inability to think in a clear, crisp fashion. Whether this is a result of, or contributor to, their distress is not clear. The type of cognitive deficit described here, however, may be reflected in their inconsistent views of themselves. That is, they experience a marked contrast between their characterization of their habitual state--which is distressed--and their perception of how they feel at any given moment--which was relatively normal.

Monroe reported that poor sleepers differed from good sleepers immediately before and during sleep on a variety of autonomic measures, including temperature, heart rate, pulse volume and vasoconstriction rate. Although most of these observations were consistent with the concept of heightened autonomic activity in poor sleepers, the higher skin resistance (usually a sign of relaxation) in the poor sleepers suggests that the issue is more complex. In the present study there was a trend toward an increased temperature during the first two hours of sleep which (during that period, at least) was not due to increased wakefulness. This was similar to Monroe's finding of a mean temperature increase of 0.34°F in "poor sleepers" at sleep onset. It is possible that this sign of physiological activation corresponds to the seemingly puzzling finding that at bedtime insomniacs reported being less tired on the Daily Sleep Questionnaire.

In summary, insomniacs differ from normals in ploygraphically-defined sleep, perception of their habitual but not immediate condition, subjective level of arousal during sleep, and cognitive function when awake.

Proposed Course:

We plan to pursue the finding of impaired semantic memory processes in these patients, as well as the observation of alterations in core temperature in the patients.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00425-08 LCS

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Peripheral and Central Catecholamines in Hypertension and Stress

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Juan M. Saavedra, Medical Officer, Section on Pharmacology, LCS, NIMH

See Attached Sheet

COOPERATING UNITS (if any)

See Attached Sheet

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Pharmacology

INSTITUTE AND LOCATION

NIMH ADAMHA NIH Bethesda, Maryland 20205

TOTAL MAN-YEARS:

5.0

PROFESSIONAL:

5.0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We studied the role of brain and peripheral catecholamines, and other biogenic amines in the central regulation of cardiovascular function and during the stress response.

Adrenal catecholamine synthesis is under control of dietary sodium in genetic Dahl hypertensive rats and under central control from specific brain nuclei. One of these, the fastigial nucleus of the cerebellum, regulates the secretion of catecholamines from the adrenal gland.

We have localized specific brain stem and hypothalamic nuclei which participate in neurogenic and salt sensitive hypertension.

In addition, by the combination of RIA and HPLC methods, we studied the prostanoid profile in specific brain regions and pituitary lobes of the rat.

Names, Laboratory and Institute Affiliations, and Titles of All Other Professional Personnel Engaged on the Project:

Others: Fernand Dray, Unit Chief, Pasteur Institute, Paris, France
 Kyriaki Geroziessis, Established Investigator, Pasteur Institute, Paris, France
 Donald Reis, Professor of Neurology, Cornell University, New York
 Markku Koulo, Visiting Fellow, LCS, NIMH
 Markku Linnoila, Clinical Director of Clinical Studies, National Institute of Alcohol Abuse and Alcoholism

Project Description:

Objectives: To study the role of central and peripheral catecholamines, other biogenic amines and prostaglandins in hypertension and stress.

Methods Employed: Neuroanatomical, surgical, biochemical (HPLC, RIA, radioenzymatic).

Major Findings:

Electrical stimulation of the fastigial nucleus of the cerebellum results in release of both adrenaline and noradrenaline from the rat adrenal medulla, and of noradrenaline from the peripheral sympathetic nerves.

There is increased synthesis of adrenal catecholamines (as evidenced by increased tyrosine hydroxylase activity and increased catecholamine levels) in the genetic, salt sensitive (Dahl) rats. Both dietary sodium and genetic factors play a role in the enhanced peripheral sympathetic activity in this model.

There is evidence for two brain PNMT systems, one in the brain stem and another in the hypothalamus, which are anatomically separated.

Alterations on brain and pineal catecholamines and PNMT are present in neurogenically hypertensive rats. Peripheral adrenaline can be taken up by sympathetic nerves in the pineal gland, where it could influence nor-adrenaline release.

The prostanoid profile brain areas and the pituitary gland showed the presence of PGD₂, PGF_{2α}, PGE₂ and 6-Keto PGF_{1α} in relatively large quantities.

Significance to Biomedical Research: We have established that the brain controls the activity of the peripheral sympathetic system, partially through specific nuclei in the hypothalamus, brain stem and cerebellum. These findings allow a localized biochemical study of discrete brain areas in conditions of altered peripheral sympathetic activity, such as hypertension and stress. It is hoped that these studies will help to clarify the nature of the brain biochemical systems more important to the stress response and the cardiovascular control. This in turn will provide an opportunity for future development of

specific drugs to be used in the treatment of diverse psychosomatic illnesses related to pathological stress or to disorders of cardiovascular regulation.

Proposed Course of Project: The combination of radioenzymatic assays, RIA and HPLC will allow the study of biogenic amine turnover and prostanoid profiles in selected brain areas and pituitary gland of stressed and hypertensive animals, with the hope to advance in the understanding of the reciprocal interactions between those systems.

Publications:

Del Bo, A., Ross, C.A., Fernandez-Pardal, J., Saavedra, J.M., and Reis, D.J.: Fastigial stimulation in rats releases adrenomedullary catecholamines. Amer. J. Physiology 244:801-809, 1983.

Gerozissis, K., Saavedra, J.M., and Dray, F.: Prostanoid profile in specific brain areas, pituitary and pineal gland of the male rat. Influence of experimental conditions. Brain Res. 279:133-139, 1983.

Saavedra, J.M. and Alexander, N.: Catecholamines and phenylethanolamine N-methyltransferase in selected brain nuclei and in the pineal gland of neurogenically hypertensive rats. Brain Res. 274:388-392, 1983.

Saavedra, J.M., Del Carmine, R., McCarty, R., Guicheney, P., Weise, V., and Iwai, J.: Increased adrenal catecholamines in salt-sensitive genetically hypertensive Dahl rats. Amer. J. Physiol. 245:H762-H766, 1983.

Saavedra, J.M., Fernandez-Pardal, J., Ross, C., and Reis, D.: Dissociation between hypothalamic catecholamine levels and epinephrine forming enzyme activity after midbrain hemitranssections in the rat. Brain Res. 276:367-371, 1983.

Saavedra, J.M., Fernandez-Pardal, J., Tordal, T., Reis, D., and Ross, C.: Dissociation between rat hypothalamic and brain stem PNMT after stress and between hypothalamic catecholamines and PNMT after midbrain hemitranssections. In: Catecholamines and Other Transmitters in Stress, Usdin, E., and Kvetnansky, R., (eds.), in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00428-05 LCS

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Protein Carboxyl Methylation: A Post Translational Modifier of Protein Function

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Juan M. Saavedra, Medical Officer, Section on Pharmacology, LCS, NIMH

None

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Pharmacology

INSTITUTE AND LOCATION

NIMH ADAMHA NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

1.0

PROFESSIONAL:

1.0

OTHER

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Enzymatic carboxyl methylation of proteins is a post translational modification which reduces negative charges and alters their secretory structure and properties. It is associated with exocytotic release. Enzyme activity is very high in the posterior pituitary gland, where it is associated with methylation of neurophysins, and in the brain.

We have found at least three still unidentified proteins, in addition to neurophysins contained within axons of brain origin in the pituitary gland.

Determination of enzyme activity in hypothalamic nuclei demonstrate high activity in the magnocellular nuclei associated with production of vasopressin, oxytocin and neurophysins.

Project Description:

Objective: To study the relationship between enzymatic carboxyl methylation and exocytotic release in pituitary gland and brain nuclei, and to characterize newly-detected endogenous methyl acceptor proteins in pituitary gland.

Methods Employed: Enzymatic, pharmacologic, gel electrophoresis, HPLC, RIA.

Major Findings: Significant carboxylmethylation activity occurs in hypothalamic nuclei associated with neurosecretion and in the posterior pituitary. Alterations of endogenous methyl acceptor proteins occur in two examples of increased exocytosis and hormone release: dehydration (posterior pituitary) and stress (adrenal medulla).

Significance to Biomedical Research: Carboxyl methylation, by regulating the exocytotic process, may be an important factor in hormone release. Evidence has been presented to associate alterations of carboxyl methylation of neurophysins and other proteins in acute and chronic dehydration.

Proposed Course of Project: We will attempt to correlate alterations in hormone release, especially dehydration and stress, with alterations in carboxyl methylation of specific proteins in pituitary gland and discrete brain nuclei.

Publications:

Kloog, Y., and Saavedra, J.M.: Protein carboxylmethylation in intact rat posterior pituitary lobes in vitro. J. Biol. Chem. 258:7129-7133, 1983.

Saavedra, J.M., Kloog, Y., Chevillard, C., and Fernandez-Pardal, J.: High protein carboxylmethylase activity and low endogenous methyl acceptor proteins in posterior pituitary lobe of rats lacking neurophysin-vasopressin (Brattleboro rats). J. Neurochem. 41:195-200, 1983.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00433-04 LCS

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Role of Neuropeptides in Neuroendocrine Regulation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Juan M. Saavedra, Medical Officer, Section on Pharmacology, LCS, NIMH

See Attached Sheet

COOPERATING UNITS (if any)

See Attached Sheet

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NIMH ADAMHA NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

5.0

PROFESSIONAL:

5.0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project studies the interrelationships between several neuropeptides (vasopressin, angiotensin II, corticotropin releasing factor and somatostatin) and their role in neuroendocrine regulation. We use animal models of genetic diseases: the Brattleboro rat, which in its homozygous state cannot produce vasopressin, and the spontaneously hypertensive rat.

A relationship between angiotensin II, somatostatin and vasopressin can clearly be shown in individual brain nuclei and pituitary gland of Brattleboro rats.

Angiotensin II selectively stimulates glucose utilization in the subfornical organ and the posterior pituitary.

We developed new techniques for the localization of angiotensin II receptors in brain nuclei and peripheral organs.

We have found that two pituitary neuropeptides are present in fibers originated in the brain: corticotropin-releasing factor (in posterior lobe and intermediate lobe) and somatostatin (in intermediate lobe only). Specific roles for both peptides are postulated in the intermediate pituitary lobe.

Names, Laboratory and Institute Affiliations, and Titles of All Other Professional Personnel Engaged on the Project:

Others: Fernand Dray, Head, Unit on Radioimmunoassay, Institute Pasteur, Paris, France
Catherine Rougeot, Chemist, Institute Pasteur, Paris, France
Henry Holcomb, Clinical Associate, LPP, NIMH
Masako Kadekaro, Visiting Scientist, LCM, NIMH
Louis Sokoloff, Chief, LCM, NIMH

Project Description:

Objectives: To study the functions and interactions of vasopressin, angiotensin II, corticotropin-releasing factor and somatostatin in brain nuclei, posterior and intermediate pituitary lobes, and their role in neuroendocrine regulation.

Methods Employed: Neuroanatomical, biochemical, RIA, HPLC.

Major Findings: Brattleboro rats show increased glucose utilization in subfornical organ (SFO) and the posterior pituitary. Those changes are related to angiotensin II stimulation of SFO receptors.

Angiotensin II receptors have been localized by autoradiography with computerized densitometry and mapped throughout the brain and in peripheral organs.

Corticotropin-releasing factor in intermediate and posterior pituitary lobes is contained within nerve fibers of brain origin. Somatostatin is localized in the intermediate lobe in nerves of brain origin. In this tissue, somatostatin interacts with catecholamines and may participate in regulation of α -MSH release.

Significance to Biochemical Research: These studies will partially clarify the role of vasopressin, angiotensin II, corticotropin-releasing factor, and somatostatin in neuroendocrine regulation.

Proposed Course of Project: We plan to study further the interactions between those neuropeptides, with particular emphasis in correlations between neuroendocrine states and neuropeptide molecular form profiles.

Publications

Chevillard, C., and Saavedra, J.M.: Angiotensin-converting enzyme (Kininase II) in pituitary gland of spontaneously hypertensive rats. Regulatory Peptide 5:333-341, 1983.

Chevillard, C., and Saavedra, J.M.: Selective increase of angiotensin-converting enzyme activity in discrete extrahypothalamic areas of Brattleboro rats. Brain Research 272:283-290, 1983.

Correa, F.M.A., and Saavedra, J.M.: High histamine levels in specific hypothalamic nuclei of Brattleboro rats lacking vasopressin. Brain Research 276:247-252, 1983.

Correa, F.M.A.; and Saavedra, J.M.: Somatostatin inhibits the isoproterenol-stimulated adenylate cyclase in the intermediate lobe of the male rat pituitary. Neuroendocrinology 37:284-287, 1983.

Correa, F.M.A., and Saavedra, J.M.: Radioimmunoassay of met-enkephalin in microdissected area of parafornix dehyde-fixed rat brain. Life Sciences 34:809-817, 1984.

Kadekaro, M., Gross, P.M., Sokoloff, L., Holcomb, H.H. and Saavedra, J.M.: Elevated glucose utilization in the subfornical organ and pituitary neural lobe of the Brattleboro rat. Brain Research 275:189-193, 1983.

Mendelsohn, F.A.O., Aguilera, G., Saavedra, J.M., Quirion, R., and Catt, K.J.: Characteristics and regulation of angiotensin II receptors in pituitary, circumventricular organs and kidney. Clin. and Exper. Theory and Practice A5 (7 and 8):1081-1097, 1983.

Mendelsohn, F.A.O., Quirion, R., Saavedra, J.M., Aguilera, G., and Catt, K.J.: Autoradiographic localization of angiotensin II receptors in rat brain. Proc. Nat. Acad. Sci. (USA) 81:1575-1579, 1984.

Miyazaki, K., Saavedra, J.M., Cote, T.E., and Keabian, J.W.: Desensitization of the D-2 dopamine receptor and the β_2 -adrenoreceptor in the intermediate lobe of the rat pituitary gland. Neurochemistry International 5:803-810, 1983.

Saavedra, J.M.: Vasopressin and somatostatin in specific hypothalamic nuclei: interaction in stress, genetic hypertension and diabetes insipidus. In: Catecholamines and Other Transmitters in Stress, Usdin, E., and Kvetnansky, R., eds., in press.

Saavedra, J.M., Rougeot, C., Chevillard, C., and Dray, F.: High, vasopressin reversible, immunoreactive somatostatin in specific hypothalamic nuclei of rats with diabetes insipidus (Brattleboro rats). Brain Research 277:23-30, 1983.

Saavedra, J.M., Rougeot, C., Culman, J., Israel, A., Niwa, M., Tonon, M.C., Vaudry, H., and Dray, F.: Decreased corticotropin-releasing factor-like immunoreactivity in rat intermediate and posterior pituitary after stalk section. Neuroendocrinology, in press.

Saavedra, J.M., Rougeot, C., and Dray, F.: Selective decrease in immunoreactive somatostatin in rat intermediate pituitary lobe after stalk section. Neuroendocrinology 37:164-165, 1983.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00434-03 LCS

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cellular Mechanisms of ACTH Secretion from Mouse Pituitary Tumor Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Julius Axelrod, Chief, Section on Pharmacology, LCS, NIMH

See Attached Sheet

COOPERATING UNITS (if any)

See Attached Sheet

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Pharmacology

INSTITUTE AND LOCATION

NIMH ADAMHA NIH Bethesda, Maryland 20205

TOTAL MAN-YEARS

3.0

PROFESSIONAL:

3.0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The cellular mechanisms involved in adrenocorticotropin (ACTH) release were studied in a tumor cell line of the mouse anterior pituitary (AtT-20/D16-16). Forskolin, an effective activator of adenylate cyclase increases cAMP accumulation, cAMP-dependent protein kinase activity and ACTH release with equal potency. The phosphorylation of several endogenous proteins is altered by forskolin acting through cAMP-dependent protein kinase. These data support a role for cAMP as a second messenger in the receptor-mediated release of ACTH.

Somatostatin (SRIF) is a potent inhibitor of ACTH release from AtT-20 cells. This peptide blocks hormone and forskolin-stimulated cyclic AMP formation and ACTH release by activating a guanine nucleotide inhibitory protein (N_i). This proposal is supported by experiments employing pertussis toxin. This agent catalyzes the ADP-ribosylation of N_i in AtT-20 cell membranes. Pertussis toxin blocks the ability of SRIF to inhibit corticotropin releasing factor (CRF) and forskolin-stimulated cAMP accumulation and ACTH release by inactivating N_i.

SRIF receptors can become desensitized on AtT-20 cells. Pretreatment with SRIF reduces the subsequent ability of SRIF to inhibit CRF and forskolin-stimulated cAMP accumulation and ACTH. This desensitization may involve an uncoupling of N_i and SRIF receptors.

Names, Laboratory and Institute Affiliations, and Titles of Co-principal Investigator and All Other Professional Personnel Engaged on the Project.

Others: Terry D. Reisine, Senior Staff Fellow, LCS NIMH
 Alberto Luini, Visiting Associate, LCS NIMH
 John Kebabian, Chief, Biochemical, Neuropharmacology Section, NINCDS
 Ronald Sekura, Research Chemist, DMI NICHD
 Andrew Hoffman, Department of Medicine, Stanford University
 Medical Center, Stanford, California
 Fountaine C. Brown, Guest Researcher, LCS NIMH
 Jean Deupree, Guest Researcher, LCS NIMH
 Edward Redgate, Guest Researcher, LCS NIMH
 Eva Mezey, Visiting Fellow, LCB NIMH

Project Description:

Objectives: To investigate the molecular events involved in ACTH release from the anterior pituitary tumor cell line, AtT-20/D16-16. Regulation of the hormone receptors linked to ACTH release will also be examined.

Methods Employed:

Cell culture: Standard procedures for culturing both tumor cells and primary monolayers.

Biochemical: Radioimmune assays for ACTH and cyclic AMP; gel electrophoresis, protein phosphorylation techniques, ADP-ribosylation of proteins employing [³²P]-NAD and pertussis toxin and cellular subfractionation.

Pharmacological: Ligand binding assays, superfusion apparatus to measure continuous ACTH and cyclic AMP release, Ca⁺⁺ flux assays, Quin-2 to examine Ca⁺⁺ mobilization.

Major Findings:

The AtT-20/D-16-16 mouse pituitary cell line is a useful system to study the molecular events involved in ACTH release since the cells are a homogeneous population of corticotrophs containing a variety of hormone receptors linked to the ACTH release process. Different hormones acting through a variety of second messenger systems affect this release process. Cyclic AMP may be a second messenger in the receptor-mediated release of ACTH. Activation of adenylate cyclase by forskolin increased cyclic AMP production, cyclic AMP-dependent protein kinase and releases ACTH. The phosphorylation of several endogenous proteins in these cells was affected by forskolin and studies will be conducted to link the activation of cyclic AMP-dependent protein kinase and the phosphorylation of these proteins with the ACTH release process. K⁺ also increases ACTH release yet does not alter cyclic AMP levels. K⁺ may evoke ACTH release by increasing Ca⁺⁺ influx and mobilization in AtT-20 cells. Experiments employing superfusion apparatus have

revealed that K^+ and forskolin stimulate ACTH release through different mechanisms.

While several hormones evoke ACTH release, the hypothalamic peptide somatostatin (SRIF) is a potent inhibitor of ACTH secretion. SRIF acts through multiple mechanisms to prevent ACTH release. Studies employing pertussis toxin have shown that SRIF acts through a guanine nucleotide inhibitory protein (N_i) to reduce the ability of secretagogues to increase cyclic AMP accumulation. SRIF also blocks K^+ -evoked ACTH release but not through a mechanism involving adenylate cyclase. An alteration in some Ca^{++} mediated event may explain SRIF's inhibition of K^+ stimulated ACTH release. AtT-20 cells can become refractory to some of SRIF's inhibitory actions and studies are in progress to determine the molecular events involved in SRIF desensitization.

Significance to Biomedical Research: The release of ACTH is an important response of the body to stress. By studying the mechanisms by which different hormones regulate ACTH secretion, it may be possible to understand at a cellular level the events involved in stress and the possible molecular abnormalities associated with chronic stress.

Proposed Course of Project: Future studies will be directed to elucidating the various intracellular events involved in ACTH release. The regulation of SRIF receptors and N_i and the molecular changes induced by chronic SRIF treatment will be examined. Attempts will be made in rats to determine whether SRIF can prevent the rise in plasma ACTH levels induced by stress.

Publications:

Axelrod, J. and Reisine, T.D.: Interaction among the stress hormones. Science, in press, 1984.

Reisine, T.: Somatostatin desensitization: Loss of the ability of somatostatin to inhibit cyclic AMP accumulation and adrenocorticotropin hormone release. J. Pharmacol. Expt. Therap. 229: 14-20, 1983.

Reisine, T. and Heisler, S.: Desensitization of beta-adrenergic receptors linked to adrenocorticotropin secretion. J. Pharmacol. Exper. Ther. 227:107-114, 1983.

Reisine, T. and Takahashi, J.: Somatostatin pretreatment desensitizes somatostatin receptors and induces supersensitivity of adenylate cyclase to forskolin and hormone stimulation in mouse anterior pituitary tumor cells. J. Neuroscience, in press.

Reisine, T.D., Zhang, Y-l, and Sekura, R.D.: Pertussis toxin blocks somatostatin's inhibition of stimulated cyclic AMP accumulation in anterior pituitary tumor cells. Biochem. Biophys. Res. Commun. 115: 794-799, 1983.

Heisler, S., and Reisine, T.: Forskolin stimulates adenylate cyclase activity, cyclic AMP accumulation and adrenocorticotropin secretion from mouse anterior pituitary tumor cells. J. Neurochem., in press.

Miyazki, K., Reisine, T. and Kebabian, J.: Cyclic AMP-dependent protein kinase activity in mouse anterior pituitary tumor cells: occurrence, activation by agents promoting cAMP-dependent ACTH secretion, and endogenous substrates. Endocrinol., submitted.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00382-10 LCS
PERIOD COVERED October 1, 1983 to September 30, 1984		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Localization and Characterization of Brain Neuropeptides		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
David M. Jacobowitz	Chief, Histopharmacology Section	LCS, NIMH
Gerhard Skofitsch	International Research Fellow (Fogarty International Center)	LCS, NIMH
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Clinical Science		
SECTION Histopharmacology		
INSTITUTE AND LOCATION NIMH, ADAMHA, Bethesda, Maryland 20205		
TOTAL MAN-YEARS: 1.7	PROFESSIONAL: 1.3	OTHER: .4
CHECK APPROPRIATE BOX(ES)		
<input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)		
<p>(1) The localization of <u>human growth hormone releasing factor (GRF)</u> immunoreactive neurons was studied in the rat brain. A dense accumulation of GRF-containing fibers was noted in the external layer of the <u>median eminence</u>. Cell bodies were observed in colchicine-treated rats. The most intensely fluorescent cluster of cells was contained in the <u>arcuate nucleus</u>. Other cells were seen on the base of the <u>hypothalamus</u>, within the median forebrain bundle, dorsal and ventral aspects of the ventromedial nucleus, zona incerta and dorsal part of the dorsomedial nucleus. These cells may influence the pulsatile release of pituitary growth hormone.</p> <p>(2) <u>Corticotropin releasing factor (CRF)</u>-like immunoreactive neurons have been identified in the rat retina by immunohistochemical methods using antisera directed against ovine and rat CRF. CRF-like immunoreactivity was observed in both <u>amacrine and ganglion cells</u> which projected fine varicose processes to the inner plexiform layer of the retina.</p> <p>(3) Treatment of newborn rats with <u>capsaicin (CAP)</u> was shown to cause a disappearance of CRF-like immunoreactive nerve fibers in the dorsal horn of the <u>spinal cord</u> (laminae I and II), the <u>spinal trigeminal nucleus</u> and tract and the <u>nucleus tractus solitarius</u>, but not in the median eminence and the central amygdaloid nucleus. Since it is well known that CAP acts selectively on primary sensory neurons of the C-fiber type, it is suggested that CRF is also located in peripheral sensory neurons, representing a novel peptidergic neuronal system, possibly involved in the modulation of transmission of peripheral nociceptive impulses, which is different from the CAP resistant hypothalamo-infundibular CRF system.</p>		

Project Description:

Objectives: 1) Localization and identification of growth hormone releasing factor (GRF)- and corticotropin releasing factor (CRF)-like immunoreactive cell bodies and nerve fibers in the central and peripheral sensory nervous system of the rat and 2) biochemical characterization of CRF-like material picked up by different antibodies directed against either ovine or rat CRF and 3) localization of growth hormone releasing factor (GRF)-like immunoreactive neurons in the brain.

Methods Employed: 1) Immunohistochemistry of GRF and CRF, 2) stereotaxically placed microinjections of retrogradely transported fluorescent dyes and 3) radioimmunoassay of CRF.

Major Findings:

(A) GRF-localization. Antisera to human GRF revealed that the highest concentrations of GRF-like immunoreactive nerve terminals were found in the external layer of the median eminence and in the pituitary stalk. Cell bodies in colchicine-treated rats were observed most prominently in the arcuate nucleus just lateral to the median eminence. Other moderately intense fluorescent cells were observed enveloping the fornix, on the base of the hypothalamus, within the median forebrain bundle, zona incerta, dorsal and ventral aspects of the periphery of the ventromedial nucleus and dorsal part of the dorsomedial nucleus.

(B) CRF-localization. 1) CRF-like immunoreactive neurons have been identified in the rat retina using antisera directed against ovine and rat CRF. CRF-like immunoreactivity was observed in both amacrine and ganglion cells which projected fine varicose processes to the inner plexiform layer of the central retina. Amacrine cells send either bistratified processes to laminae 1 and 4 or unistratified processes to laminae 1 and 4 of the inner plexiform layer, whereas ganglion cells send processes to laminae 4 and 5 of the inner plexiform layer. It is suggested that a CRF-like material may play a role in retinal function. 2) Ovine CRF-like immunoreactivity was previously identified by different authors in the dorsal horn of the spinal cord (laminae I and II), the spinal trigeminal nucleus and tract, and the nucleus tractus solitarius - areas which are rich in terminals of primary sensory neurons. Since it is well known that systemic treatment of newborn rats with capsaicin, the pungent agent of hot peppers, causes irreversible but selective destruction of peptidergic primary sensory neurons of the C-fiber type, mostly of polymodal nociceptors, the effect of capsaicin on o-CRF-like immunoreactivity was studied. Neonatal treatment of rats with capsaicin resulted in an almost complete loss of o-CRF-like immunoreactivity in the dorsal horn of the spinal cord at all levels, the spinal trigeminal nucleus and tract and the nucleus tractus solitarius suggesting that the main portion of o-CRF-like material in these regions is contained in primary afferent sensory neurons. It is suggested that a CRF-like peptide may play an important role in the modulation or in the transmission of peripheral nociceptive impulses. 3) The effect of neonatal capsaicin treatment in rats was studied with respect to other neuropeptides (substance P, somatostatin, vasoactive intestinal polypeptide, cholecystokinin, neuropeptide Y) also present in areas where CRF-like immunoreactivity was affected. As suggested from the literature, substance P,

vasoactive intestinal polypeptide and cholecystokinin were depleted by capsaicin as is CRF; whereas somatostatin and neuropeptide Y were not affected. 4) We tried to identify the source of cell bodies which send their axons to the spinal trigeminal tract by stereotaxic microinjection of retrogradely transported fluorescent dye (fast blue) in the trigeminal ganglion. Substance P, somatostatin, vasoactive intestinal polypeptide, cholecystokinin and o-CRF-like immunoreactive cell bodies were found in the trigeminal tract. Immunoreactive cell bodies of these peptides were also found in the dorsal root ganglia of the cervical spinal cord.

With respect to the different maps of ovine CRF-like immunoreactivity published at present in the literature and the appearance of a new rat-human CRF peptide, an attempt was made to characterize a variety of antibodies by biochemical methods. 1) About fifty nuclei were microdissected from rat brain. The CRF levels were determined by radioimmunoassay (RIA) and compared. For each region, four different assays were done, using antibodies directed against rat-human and ovine CRF. 2) Complete maps of immunoreactive cell bodies and terminals obtained by immunohistochemistry were prepared for each antibody. 3) RIA-displacement curves for each of the four antibodies with rat-human CRF, ovine CRF, sauvagine (a CRF-related peptide) and substance P were prepared and differences in cross-reactivity and specificity observed.

Significance to Biomedical Research and the Program of the Institute:

The demonstration of GRF cell bodies in the hypothalamus has important implications for the basic understanding of the physiologic release of pituitary growth hormone.

The basic neuroanatomical and methodological studies reported here lay the groundwork for an approach to study the role of CRF in the central and peripheral nervous system.

Proposed Course of the Project;

1) We will try to find out which CRF antibody is the most reliable to use in the rat brain.

2) Attempts are in progress to identify possible non-specific substances that CRF antibodies are reacting with, e.g. the most interesting one is whether the substance contained in sensory nerves is real CRF or a structurally similar analogue. It is important to identify this substance as it might be involved in modulation of pain perception or transmission of nociceptive impulses.

3) After having identified the best RIA for CRF, drug treatment studies and stress experiments can be undertaken.

Publications:

Jacobowitz, D.M., Schulte, H., Chrousos, G.P. and Loriaux, D.M.: Localization of GRF-like immunoreactive neurons in the rat brain. Peptides 4: 521-524, 1983.

Jacobowitz, D.M. and Olschowka, J.A.: Coexistence of bovine pancreatic polypeptide and norepinephrine in the central and peripheral nervous systems. In Chan-Palay, V. and Palay, S.L. (Eds.): Coexistence of Neuroactive Substances in Neurons. New York, John Wiley & Sons, 1984, pp. 91-112.

Skofitsch, G., Hamill, G.S. and Jacobowitz, D.M.: Capsaicin depletes corticotropin releasing factor-like immunoreactive neurons in the rat spinal cord and medulla oblongata. Neuroendocrinology, 38: 514-517, 1984.

Skofitsch, G. and Jacobowitz, D.M.: Corticotropin releasing factor-like immunoreactive neurons in the rat retina. Brain Research Bulletin, in press, 1984.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00388-08 LCS

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Interpeduncular Nucleus Afferents and Endogenous Peptides

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

David M. Jacobowitz Chief, Histopharmacology Section LCS, NIMH

Geoffrey S. Hamill Senior Staff Fellow LCS, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Histopharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

1.6

PROFESSIONAL:

1.2

OTHER:

.4

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard un-reduced type. Do not exceed the space provided.)

Forebrain and hindbrain afferents projecting to the interpeduncular nucleus (IPN) have been demonstrated in male rats by retrograde transport of fluorescent dye "fast blue" microinjected into IPN followed by intraventricular colchicine 48 hrs prior to perfusion. The most intensely labeled cells projecting to IPN were concentrated throughout the entire rostrocaudal extent of the medial habenular nuclei. Immunocytochemistry revealed small numbers of labeled medial habenular cells having substance P immunofluorescence. Additional forebrain afferents originate from septal, hypothalamic and mammillary nuclei. Hindbrain afferents projecting to IPN include the nucleus incertus, a circumscribed region overlying the dorsal tegmental nucleus. Many labeled cells in the nucleus contain leu-enkephalin-like immunofluorescence. Additional brainstem afferents include the raphe, dorsolateral tegmental nuclei and locus coeruleus.

Immunocytochemical studies of IPN reveal a wide variety of endogenous peptides and biogenic amines distributed in somata and processes in a topographic and heterogeneous pattern. Substance P, cholecystokinin, vasoactive intestinal peptide, somatostatin, leu-enkephalin, dopamine- β -hydroxylase and serotonin were observed in IPN of male rats treated with colchicine 48 hrs prior to perfusion. Immunofluorescent cell bodies and processes were distributed differentially among IPN subnuclei revealing new features of the organization.

Project Description:

Objectives: 1) To examine the distribution of forebrain and brainstem afferents projecting to the IPN by retrograde transport of fluorescent dye "fast blue" in conjunction with immunocytochemical studies examining the peptidergic content of these afferents and 2) to examine the subnuclear distribution of a variety of peptides and biogenic amines within IPN using immunocytochemical techniques.

Methods Employed: 1) Stereotaxic microinjection, 2) fluorescent microscopy and 3) immunocytochemistry.

Major Findings:

(A) For the first time, we now demonstrate afferents containing substance P and leu-enkephalin arising from cells in the medial habenula and nucleus incertus, respectively.

(B) We also demonstrate a topographic and heterogeneous subnuclear distribution of neuropeptides (leu-enkephalin, substance P, cholecystokinin, vasoactive intestinal peptide, somatostatin) and biogenic amines (DBH, serotonin) within the IPN.

(C) These findings, coupled with our prior observations of a correlation between the localization of AChE staining nerves and substance P immunoreactive nerves within the IPN and the distribution of muscarinic and α -bungarotoxin binding sites have revealed that the IPN is a remarkable heterogeneous organ within the brain.

Significance to Biomedical Research and the Program of the Institute: The IPN, a single midbrain nucleus, receives a complex distribution of both forebrain and hindbrain afferents, some of which contribute to its exogenous content of neuropeptides. In addition, a wide variety of endogenous peptides and biogenic amines are present in well defined subnuclei of IPN. These studies lay the groundwork for future studies concerning the physiological significance of the IPN.

Proposed Course of the Project: Further studies on the IPN are in progress.

Publications:

Rotter, A. and Jacobowitz, D.M.: Colocalization of substance P, acetylcholinesterase, muscarinic receptors and alpha-bungarotoxin binding sites in the rat interpeduncular nucleus. Brain Res. Bull. 12: 83-94, 1984.

Hamill, G.S., Olschowka, J.A., Lenn, N.J. and Jacobowitz, D.M.: The subnuclear distribution of substance P, vasoactive intestinal peptide, cholecystokinin, somatostatin, leu-enkephalin, serotonin and dopamine beta hydroxylase in the rat interpeduncular nucleus. J. of Comp. Neurology, in press, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
 Z01 MH 00396-06 LCS

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders)

A Study of Proteins Within the CNS by Two-Dimensional Gel Electrophoresis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

William E. Heydorn	Guest Researcher (NIGMS Fellow)	NIGMS & LCS, NIMH
David M. Jacobowitz	Chief, Histopharmacology Section	LCS, NIMH
Charles W. Scouten	Guest Researcher	LCS, NIMH
Paul J. Marangos	Chief, Unit on Neurochemistry	LCS, NIMH
Raj Narayan	Staff Neurosurgeon	SNB, NINCDS
David Klein	Chief, Section on Neuroendocrinology	LDN, NICHD

COOPERATING UNITS (if any)

Surgical Neurology Branch, NINCDS
 Laboratory of Developmental Neurology, NICHD

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Histopharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, Bethesda, Maryland 20205

TOTAL MAN-YEARS

3.4

PROFESSIONAL:

2.4

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

Using two-dimensional gel electrophoresis (2DE), an atlas was constructed showing the location and relative concentration of a number of different proteins from 25 distinct neuroanatomical regions of the male rat brain. Using this as a basis, a number of different projects were initiated. First, we have shown that the concentration of two distinct proteins is different in the medial preoptic area of male and female rats. Second, we have shown that disruption of the cholinergic innervation to the hippocampus and the occipital cortex affects the concentration of 4 different proteins. This suggests that the amounts of these polypeptides in brain are regulated by the cholinergic nervous system. Third, we have initiated a series of studies using human cortical tissue and have shown the following: A) The apparent concentration of a number of different proteins changes shortly after death, suggesting that results obtained using post-mortem human brain tissue should be interpreted with caution. B) Irradiation as a treatment for various carcinomas of the CNS apparently alters the concentration of a small number of proteins in the surrounding normal tissue. C) Different types of CNS tumors have protein patterns which are both distinct and different from that seen in normal cortical tissue. Fourth, we have succeeded in identifying a number of heretofore unidentified proteins on two-dimension gels of rat and human cortex. These include the isomers of the glycolytic enzyme enolase, glial fibrillary acidic protein, alpha and beta tubulin and a number of neurofilament proteins. Finally, we have shown that proteins from discrete nuclei in fresh brain tissue can be labeled with [³⁵S]-methionine in vitro. These proteins can then be separated by 2DE and radiolabel uptake estimated by autofluorography. These results provide an estimate of amino acid incorporation into protein during short-term tissue culture experiments.

Project Description:

Objectives: 1) Investigate differences in the medial preoptic and lateral ventromedial nuclei between male and female animals and determine which of the proteins present in these areas are influenced in concentration by the sex steroid hormones. 2) Demonstrate which proteins in cortical tissue are regulated in concentration by the cholinergic nervous system. 3) Determine which proteins in human cortical tissue change in concentration shortly after death. 4) Show whether irradiation as a treatment for CNS tumors affects any proteins in the surrounding normal cortex. 5) Determine whether the protein pattern in various types of CNS tumors differs from that seen in normal cortex. 6) Identify various proteins present on two-dimension gels using Western blots. 7) Investigate whether brain proteins can be labeled by incubation with radiolabeled amino acids and then visualized using the combination of 2DE and autofluorography.

Methods Employed: 1) Two-dimensional polyacrylamide gel electrophoresis; 2) Photochemical silver staining of proteins on polyacrylamide gels; 3) Computerized scanning densitometry of proteins on two-dimensional gels; 4) Microdissection of discrete regions of the rat brain; 5) Electrophoretic transfer of proteins to nitrocellulose paper and subsequent identification of proteins by use of specific antisera; 6) Radiolabel amino acid incorporation into proteins from fresh brain tissue using short term tissue culture techniques; 7) Autofluorography of radiolabeled proteins.

Major Findings:

(A) Using 2DE, we have identified two proteins whose concentration differs in the medial preoptic area between male and female rats. The first, identified by our nomenclature as protein number 11, has a molecular weight of 57,000 daltons and a pI of 6.3. This protein was found to be present in higher amounts in the preoptic medial nucleus (POM) in the male than in the same area of the female. Interestingly, this protein is identical to one of those in the hippocampus found to be reduced in concentration by chronic administration of the tricyclic antidepressant desmethylimipramine and elevated in concentration by chronic administration of the neurotransmitter depleting agent reserpine. The second protein differing between the male and female POM is protein 28, which has a molecular weight of 39,000 daltons and a pI of 6.1. This protein was found in higher concentrations in the female POM than in the male POM. In both cases, no difference was detected in the parietal cortex.

(B) These studies on protein differences between the male and female brain have recently been expanded by examining the effects of various hormone treatments on proteins in the POM and the lateral ventromedial nucleus (VMN-L). These two brain areas were selected for these studies because the POM is believed to regulate sexual behavior in the male, while the VMN-L performs the same function in the female. The results obtained to date demonstrate that several proteins in both of these brain areas are affected in concentration by steroid hormones. Interestingly, some of these proteins respond in the POM only in the male and in the VMN-L only in the female. These results are interesting in light of the different roles played by the POM and the VMN-L in regulating sexual behavior in the male and the female, respectively.

(C) We have embarked on a series of experiments designed to ascertain which proteins are regulated by the major neurotransmitter systems in the CNS. Since the nucleus of the tractus diagonalis (td) provides the major cholinergic input to both the hippocampus and the occipital cortex, we decided to electrolytically ablate the td and examine protein concentrations in these two brain areas using 2DE. Of the 140 proteins examined, only four were found to be altered in concentration in both the hippocampus and the occipital cortex after the td lesion. Protein 82 (molecular weight 39,000 daltons, pI 6.5) was reduced 71% in the hippocampus and 50% in the occipital cortex nine days after the lesion, while protein 109 (molecular weight 32,000 daltons, pI 6.4) was elevated 140% in the hippocampus and 130% in the occipital cortex at the same time point. Thirty five days after the lesion, the concentration of both of these proteins had returned to control levels. A third protein, designated as number 6 (molecular weight 58,000 daltons, pI 5.7), was unchanged in concentration nine days after the lesion, but was elevated 44% in the hippocampus and 27% in the occipital cortex 35 days after lesion. The fourth protein affected by the lesion, number 74 (molecular weight 39,000 daltons, pI 5.8), was significantly elevated in concentration both 9 and 35 days after lesion in the occipital cortex, but only at day 35 in the hippocampus. In all cases, no effect of the lesion was detected in the caudate nucleus, a region not receiving its cholinergic input from the td.

(D) Using a combination of 2DE, silver staining and computerized densitometry, we have initiated a study of proteins in human cerebral cortex. Samples of normal fresh frozen, post-mortem and irradiated cortex were compared quantitatively. Computerized densitometry demonstrated significant alterations in the density of several spots in the radiated and post-mortem groups as compared to the normal controls. Radiated cortex showed significant changes in only 6 spots, with half showing an increase in density and the other half a decrease in density. Post-mortem material, however, showed 20 altered spots, with 16 of these registering diminished densities. Several other proteins appeared altered in density in post-mortem tissue, but the changes failed to reach statistical significance due to the high variance in the post-mortem group.

(E) Protein patterns have been examined qualitatively from a variety of different CNS tumors. To date, a total of 50 tumors have been examined, subdivided as follows: 24 astrocytomas, 9 meningiomas, 5 pituitary adenomas, 4 medulloblastomas, 2 craniopharyngiomas, 3 juvenile astrocytomas, 1 ependymoma, 1 schwannoma and 1 choroid plexus papilloma. A qualitative assessment of the two-dimension gels from the different tumor types has revealed marked differences in all groups from that seen in control samples. It appears that each tumor has its own characteristic two-dimension pattern of protein spots. The possibility exists that this may be utilized as a method of biochemically diagnosing different forms of CNS tumors.

(F) Using a combination of co-migration with the appropriate purified protein and staining on nitrocellulose protein blots, we have identified the location of the glycolytic enzymes neuron-specific enolase (NSE) and non-neuronal enolase (NNE) on two-dimension gels generated using rat and human cortex. In the rat, NSE appears as a unique doublet spot with an average molecular weight of 44,000 daltons and a pI of 5.4. NNE in the rat is both slightly larger (49,000 daltons) and more basic (pI 6.6) than NSE. NSE corresponds to proteins number 15 and 16 in our original two-dimension gel mapping study in the rat, while NNE is protein

31. In the human, NSE appears as a single spot of molecular weight 47,000 daltons and pI of 5.2, while NNE is significantly more basic (pI 7.2) and slightly larger (49,000) than NSE. This information will prove useful in future studies identifying specific proteins on two-dimension gels and observing the effects of experimental manipulations on brain and other neuronal proteins.

(G) A method has been developed for the incorporation of [³⁵S]-methionine into proteins from freshly dissected brain tissue. Rats were killed by decapitation, the brain was rapidly removed and sectioned without freezing using a vibratome. Various hypothalamic nuclei and circumventricular organs were removed by microdissection and incubated in a media containing [³⁵S]-methionine for six hours. Proteins within these tissue samples were then separated by two-dimensional polyacrylamide gel electrophoresis. The gels were stained with silver, photographed, dried and exposed to autoradiographic film. The results indicate that 1) this technique will prove useful in examining the uptake of radiolabeled amino acids into proteins from discrete nuclei and other brain areas, and 2) there is considerable regional heterogeneity in the incorporation of [³⁵S]-methionine into proteins by the different brain areas examined.

Significance to Biomedical Research and the Program of the Institute: The identification of specific proteins that differ both in baseline concentration and in sensitivity to steroid hormones in discrete hypothalamic nuclei between the male and the female provides a basis from which to further study differences between the sexes that may exist in the central nervous system. Demonstrating that lesions of the cholinergic nervous system can affect protein concentrations in cortex and hippocampus may prove to be a model for the study of Alzheimer's disease. This condition, characterized by a range of different neurological symptoms, including a progressive deterioration of cognitive functions indistinguishable from senile dementia but beginning as young as 40 years of age, has been linked to a deficit in cholinergic neurotransmission. The possibility exists that the proteins found to be altered in concentration by a lesion of the cholinergic nervous system may also be altered in Alzheimer's disease. Our studies on normal and diseased human cortex are providing a new biochemical method to examine CNS tumors. The initial studies have shown that proteins in normal cortex undergo dramatic changes shortly after death, with the result that studies done with post-mortem human brain tissue must be carefully interpreted when attempting to extrapolate back to the in vivo situation. The qualitative tumor work completed to date shows that proteins within tumors are significantly altered from the normal state. The significance of this to the etiology of tumor development remains to be investigated. Our studies identifying specific proteins on two-dimension gels are essential to an overall framework of investigating experimental manipulation on the concentration of individual proteins. As more proteins become identified using these techniques, we will be better able to interpret changes caused by both specific experimental manipulation and neuropathological conditions. Developing a method to study the uptake of radio-labeled amino acids will provide the basis for investigating turnover of individual proteins both in vitro and in vivo.

Proposed Course of the Project;

1) Expand on differences already noted between various hypothalamic nuclei of the male and female brain by using as a model the Zucker fat rat, an animal

incapable of sexual reproduction. Proteins found to be different in the Zucker fat rat will be compared to those differences already obtained between male and female brains with the goal of identifying those proteins possibly involved in sexual reproduction.

2) Investigate the possibility that one or more of the proteins found to be affected in concentration by lesions of the nucleus of the tractus diagonalis may also be altered in patients suffering from Alzheimer's disease.

3) Expand studies on specific neurotransmitters by examining the effect of lesions of the noradrenergic and serotonergic nervous systems on brain areas innervated by these transmitter systems with the goal of identifying proteins whose concentrations in brain may be regulated by these neurotransmitters.

4) Subcellular fraction of tissue samples prior to 2DE with the goal of correlating proteins visible on two-dimension gels with particular subcellular fractions. Such information will prove useful when attempting to interpret results obtained after other experimental manipulations.

5) Continue studies on human cortical tissue. These studies will focus in three main directions: a) processing of more samples of various tumor types (and controls) for analysis, b) quantitative analysis of proteins present on two-dimension gels generated using tumor tissue, and c) fractionation of tissue samples prior to 2DE so that proteins visible can be correlated with cellular subfractions.

6) Identification of proteins on two-dimension gels using a combination of co-migration with purified protein standards and cross-reactivity with specific antisera on Western blots.

7) Further investigation of radiolabeled amino acid incorporation into protein both in vitro and in vivo.

Publications:

Heydorn, W.E., Creed, G.J., Goldman, D., Kanter, D., Merril, C.R. and Jacobowitz, D.M.: Mapping and quantitation of proteins from discrete nuclei and other areas of the rat brain by two-dimension gel electrophoresis. J. Neurosc. 3: 2597-2606, 1983.

Gold, M.A., Heydorn, W.E., Creed, G.J. and Jacobowitz, D.M.: Sex differences in specific proteins in the preoptic medial nucleus of the rat hypothalamus. Neuroendocrinology 37: 470-472, 1983.

Heydorn, W.E., Creed, G.J. and Jacobowitz, D.M.: The effect of desmethylimipramine and reserpine on the concentration of specific proteins in the parietal cortex and the hippocampus of rats as analyzed by two-dimensional gel electrophoresis. J. Pharmacol. Exp. Ther., in press.

Gold, M.A., Heydorn, W.E., Creed, G.J., Weller, J.L., Klein, D. and Jacobowitz, D.M.: In vitro [35 S]-methionine labeled protein synthesis in microdissected discrete brain areas: Marked regional differences revealed by two-dimensional gel electrophoresis. Electrophoresis, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00397-06 LCS

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Neurophysiological Effects of Peptides

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

David M. Jacobowitz Chief, Histopharmacology Section LCS, NIMH

Toshio Ohhashi Guest Researcher LCS, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Histopharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, Bethesda, Maryland 20205

TOTAL MAN-YEARS

1.3

PROFESSIONAL

1.1

OTHER

.2

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided)

In order to study the physiological significance of the coexistence of pancreatic polypeptide and norepinephrine (NE) in peripheral noradrenergic nerves, the effects of pancreatic polypeptides of several species were tested on the isolated rat vas deferens. Neuropeptide Y (NPY) was also studied because of its sequence homology to the pancreatic polypeptides. The contractile responses, which were mediated predominantly by activation of noradrenergic nerves following electrical stimulation, were inhibited by bovine pancreatic polypeptide (BPP), human pancreatic polypeptide (HPP), avian pancreatic polypeptide (APP) and NPY in a dose-dependent manner using a constant flow bath. The decreasing order of the inhibitory responses was as follows: BPP=HPP>NPY>APP. The inhibitory responses produced by BPP and HPP lasted more than 1 hr and displayed a marked tachyphylaxis. In contrast, the inhibitory effects induced by NPY and APP usually returned to the control level after 20-30 min and had minimal tachyphylaxis. The inhibitory action of NPY was still present during α -adrenergic blockade. Contractions produced by a single submaximal dose of exogenous NE in unstimulated preparations were not affected by pretreatment with NPY. The amplitude of contractions was partially reduced 1 min after pretreatment with BPP or HPP; recovery occurred about 15 min after peptide pretreatment in a constant flow bath. These results suggest that an NPY receptor exists presynaptically in the rat vas deferens and that stimulation of the receptor by NPY inhibits the release of NE from noradrenergic nerves. It is concluded that both BPP and HPP act by a presynaptic inhibitory mechanism on noradrenergic nerve terminals and also have a nonspecific inhibitory action on smooth muscle cells.

Project Description:

Objectives: This work is an attempt to study the possible physiological significance of the coexistence of pancreatic polypeptide (or NPY) within noradrenergic nerves. Since PP-immunoreactivity and NE are colocalized in noradrenergic nerves of the rat vas deferens, this preparation was used to study the effects of bovine, human and avian pancreatic polypeptide and NPY on contraction of the electrically stimulated muscle. In addition, the interaction of these peptides with NE were studied on the non-stimulated vas deferens.

Methods Employed: Vas deferens stimulation in an isolated bath and recording of isometric tension with a Grass Instrument.

Major Findings:

(A) Transmural stimulation of the whole rat vas deferens was markedly reduced by 1.1×10^{-7} M BPP and HPP and lasted for about 1 hr. APP, in contrast, caused a slight inhibition of contractions caused by electrical stimulation. NPY (1.1×10^{-6} M) caused a marked reduction of the contractions which lasted about 30 min.

(B) Tachyphylaxis rapidly developed following BPP and HPP, since the same concentration caused no inhibition of contractions about 1-1.5 hr after the initial injection of the peptide. A second dose of NPY 30 min later, also produced a smaller inhibition of contraction (55% of control), which recovered sooner than the previous administration.

(C) The addition of NE (5×10^{-6} M) to the organ bath, without electrical stimulation, caused a rapid contraction that was not blocked by tetradotoxin. HPP (3.4×10^{-7} M) caused slight or no contraction in nonstimulated preparations. Contractions produced by NE 1 min after infusion of HPP were reduced to about 80% of the control contractions. NPY (1.1×10^{-6} M), at a concentration which caused marked inhibition of contractions produced by stimulation, had no inhibitory effect on the contractions developed by a single dose of NE.

(D) These results suggest that: (1) The contractile responses which were mediated predominantly by activation of post ganglionic noradrenergic nerves were inhibited by BPP, HPP, APP and NPY. (2) The decreasing order of the inhibitory responses was BPP=HPP>NPY>APP. (3) The inhibitory responses produced by BPP and HPP lasted more than 1 hr and showed a marked tachyphylaxis. In contrast, the inhibitory effects induced by NPY and APP usually returned to the control level at 20-30 min and were slightly tachyphylactic. (4) Contractions produced by exogenous NE in the preparations without electrical stimulation were not affected by NPY. (5) The long lasting effect observed following administration of these peptides in the stimulated preparation, while not seen in the resting vas deferens, suggests that a presynaptic localization of action is involved.

Significance to Biomedical Research and the Program of the Institute: Our recent observations that the class of pancreatic polypeptide (and NPY) coexist in adrenergic nerves in the brain and peripheral organs (especially blood vessels),

suggests a possible clinical relevance, particularly in problems of hypertension. The present work is an attempt to study the possible physiological significance of the coexistence of PP within noradrenergic nerves. This work suggests that this class of peptides may play a significant role in regulating prolonged release of NE from the peripheral adrenergic nerve terminals.

Proposed Course of the Project: Further studies to evaluate the influence of this class of peptides on the release of NE, and vice versa, will be pursued.

Publications:

Ohhashi, T. and Jacobowitz, D.M.: The effects of pancreatic polypeptides and neuropeptide Y on the rat vas deferens. Peptides 4: 381-386, 1983.

Diz, D.I. and Jacobowitz, D.M.: Cardiovascular effects of intrahypothalamic injections of α -melanocyte stimulating hormone. Brain Res. 270: 265-272, 1983.

Diz, D.I., Vitale, J.A. and Jacobowitz, D.M.: Increases in heart rate and blood pressure produced by injections of dermorphin into discrete hypothalamic sites. Brain Res. 294; 47-57, 1984.

Diz, D.I. and Jacobowitz, D.M. Effects of adrenalectomy, propranolol and atropine on the increase in heart rate induced by injection of dermorphin in the rat anterior hypothalamic nucleus. Brain Res. 293: 196-199, 1984.

Diz, D.I. and Jacobowitz, D.M.: Cardiovascular effects produced by injections of thyrotropin-releasing hormone in specific preoptic and hypothalamic nuclei in the rat. Peptides, in press, 1984.

Diz, D.I. and Jacobowitz, D.M.: Cardiovascular effects of discrete intrahypothalamic and preoptic injections of bradykinin. Brain Research Bull., in press, 1984.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00401-19 LCS

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Peripheral Noradrenergic Function

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Irwin J. Kopin	Scientific Director	NINCDS NIH
Kenneth L. Kirk	Research Chemist	NIAAD NIH
David C. Jimerson	Staff Psychiatrist	LCS NIMH
David Lozovsky	Guest Worker	LCS NIMH
Virginia K. Weise	Chemist	LCS NIMH
Isamu Yamaguchi	Guest Worker	LCS NIMH
Giora Feuerstein	Guest Worker	LCS NIMH
Zofia Zukowska-Grojec	Visiting Fellow	LCS NIMH
Mohamed A. Bayorh	Visiting Fellow	LCS NIMH

COOPERATING UNITS (if any)

Chuang C. Chiueh	Special Expert	OD NINCDS
David Goldstein	Senior Investigator	NHLBI

Laboratory of Bio-Organic Chemistry, NIAAD, NINCDS-OD.

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Medicine

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

3.5

PROFESSIONAL:

3.0

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The objectives of this project are to elucidate biochemical mechanisms controlling the synthesis, storage, release, action and termination of action of norepinephrine in the adrenergic neurons and how to assess these in the intact animal. 6-Fluoronorepinephrine is a substitute transmitter for norepinephrine and 6-fluorodopamine can replace dopamine in brain. Use of 6-fluorodopa as a precursor for these false transmitters is being evaluated. By blocking extra-junctional α_2 -adrenoceptors the concentration of norepinephrine at α_1 -adrenoceptors in the sympathetic neuronal-vascular smooth muscle junction has been estimated at between 10^{-8} and 10^{-6} M depending on the frequency of neuronal stimulation.

Objectives: To evaluate neurotransmitter function in animals and patients it is important for the understanding of brain function to determine the sites and rates of utilization of the substance involved in transmission of impulses at synapses in the brain and ganglia and at peripheral neuroeffector junctions.

Methods Employed: Pithed rats are used to study effects of sympathetic stimulation on norepinephrine infusion on blood pressure, heart rate, and plasma catecholamine levels. The effects on these various drugs and hormones are examined. Blood flow and cardiac output are measured with cobalt-labelled microspheres. A potential means for examining neurotransmitter function in intact humans involves the use of ^{18}F -labelled compounds which can act as (or be converted to) substances which are selectively taken up and retained at the same sites as a normal endogenously formed neurotransmitter combined with positron emission tomography (PET) scanning.

Major Findings: After developing techniques for separation and assay of fluorinated catecholamines (Drs. Chueh, Kirk and Kopin) demonstrated 6-fluoro-dopamine (6-F-DA) was to be selectively taken up into peripheral sympathetic neurons and converted to 6-fluoro-norepinephrine and is released by sympathetic nerve stimulation. Furthermore, the turnover rate of 6-F-NE is the same as that of ^3H -NE, which indicates that the fluorinated derivative can be considered a valid tracer molecule for endogenous norepinephrine. Plans are in progress for the production and use of 6- ^{18}F -DA for use in study of peripheral sympathetic function. Studies with 6-fluoro-DOPA have been initiated to establish its usefulness as a precursor for studying brain catecholamines in humans.

Previously reported studies from this laboratory have shown that after administration of drugs which selectively block α_1 -adrenoceptors, the pressor responses to stimulation of sympathetic outflow from the spinal cord of pithed rats are more effectively inhibited than are responses to administered NE. After α_2 -adrenoceptor blocking agents, the pressor responses to administered NE are inhibited more effectively than responses to stimulation. On the basis of these observations, it was proposed that α_1 -adrenoceptors are mainly intrajunctional whereas α_2 -adrenoceptors are located at sites outside the neuroeffector junction. By blocking α_2 -adrenoceptors, the role of α_2 -adrenoceptors in elevating blood pressure could be studied in pithed, adrenalmedullectomized rats treated with sufficient yohimbine to block α_2 -adrenoceptors. During sympathetic stimulation there is a direct linear relationship between the measurement in blood pressure and the logarithm of the increment in plasma norepinephrine. Similarly, during infusion of norepinephrine, the increment in blood pressure parallels the logarithm of the increment in plasma norepinephrine, but the norepinephrine levels attained during infusion must be about 10-fold higher than during stimulation to attain the same blood pressure. Treatment with DMI shifts the linear relationships between plasma norepinephrine and blood pressure by about three-fold, but in opposite directions so that during infusion lower levels of norepinephrine are required to attain the same pressor response, whereas during stimulation, a given pressor response is associated with three-fold higher plasma norepinephrine levels. The interpretation of these results leads to the conclusion that norepinephrine levels at the neuroeffector junction are the geometric mean of those found in plasma during stimulation and norepinephrine infusion. (Drs. Kopin, Zukowska-Grojec, Bayorh and Goldstein). About 2/3 of released norepinephrine is inactivated by neuronal reuptake.

During continuous sympathetic stimulation in pithed rats, the evoked pressor response slowly declines. Cobalt-57 labelled microspheres were used to measure cardiac output, organ blood flow and vascular resistance during stimulation-induced responses in demedullated, yohimbine-pretreated rats and contrasted with effects seen with similar blood pressure increases during continuous 1-norepinephrine infusion. The decline of MPB during constant stimulation results from a decrease in cardiac output. Opposite effects of stimulation and norepinephrine on regional blood flow and vascular resistance may indicate differences in accessibility of circulating vs. endogenously released norepinephrine to adrenoceptors in various vascular beds (Drs. Zukowska-Grojec, Bayorh and Kopin).

Significance to Biomedical Research and to the Program of the Institute:

Norepinephrine is the neurotransmitter released for sympathetic nerves and some neurons in brain. Understanding its formation, disposition, metabolism and action are fundamental to defining its rate in disease states and during drug action.

Proposed Course: The principle investigator and several of the other investigators in this study have been transferred to the NINCDS. Portions of this project will terminate as an NIMH project, but others may remain as collaborative efforts.

Publications:

Chieuh, C.C., Zukowska-Grojec, Z., Kirk, K.L. and Kopin, I.J.: 6-Fluorocatecholamines as false adrenergic neurotransmitters. *J. Pharmacol. Exp. Ther.* 225:529-533, 1983.

Angus, J.A., Bobik, A., Jackman, G.L.P., Kopin, I.J. and Korner, P.I.: Role of autoinhibitory feed-back in cardiac sympathetic transmission assessed by simultaneous measurements of changes in ³H-efflux and atrial rate in guinea-pig atrium. *Br. J. Pharmacol.* 81:201-214, 1984.

Kopin, I.J.: Plasma catecholamines as an index of a neuroendocrine response. In Shah, N.S. and Donald, A.G. (Eds.): Psychoneuroendocrine Dysfunction. New York, Plenum Publishing Corporation, 1984, pp. 157-171.

Kopin, I.J., Zukowska-Grojec, Z., Bayorh, M.A. and Goldstein, D.S.: Estimation of in vivo norepinephrine concentrations at vascular neuroeffector junctions. *Naunyn Schmiedeberg's Arch Pharmacol*, (1984)325:298-305.

Goldstein, D.S., McCarty, R., Polinsky, R.J. and Kopin, I.J.: Relationship between plasma norepinephrine and sympathetic neural activity. *Hypertension* 5: 552-559, 1983.

Feuerstein, G., Zukowska-Grojec, Z., Bayorh, M.A., Kopin, I.J. and Faden, A.I.: Leukotriene D₄-induced hypotension is reversed by thyrotropin-releasing hormone. Prostaglandins. 711-724, 1983.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00402-12 LCS

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

CNS Regulation of Autonomic and Endocrine Function

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Irwin J. Kopin	Scientific Director	NINCDS
Alan I. Faden	Chief, Neurobiology Research Unit	USUHS
Giora Feuerstein	Guest Worker	LCS NIMH
Andreas Pfeiffer	Guest Worker	LCS NIMH
Chuang Chiueh	Sr. Staff Fellow	LCS NIMH
Miklos Palkovits	Visiting Scientist	LCB NIMH
Zofia Zukowska-Grojec	Visiting Fellow	LCS NIMH
Mohamed Bayorh	Visiting Fellow	LCS NIMH
Robert Zerb	Sr. Staff Fellow	LCS NIMH

COOPERATING UNITS (# any)

David Jacobowitz	Chief, Sec. on Histopharmacology	LCS NIMH
Drori Ben-Ishay	Assoc. Prof. Medicine, Hadassah Hosp. Israel	

Cooperating Units: Sections on Histopharmacology and Pharmacology; LCS:

LAB/BRANCH Dept. of Neurobiology, USUHS, Beth. Md.; Hadassah Hosp. Jerusalem, Laboratory of Clinical Science Israel; LCB

SECTION

Section on Medicine

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

2.5

PROFESSIONAL:

2.5

OTHER:

0

CHECK APPROPRIATE BOX(ES)

(a) Human subjects (b) Human tissues (c) Neither

(a1) Minors

(a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The purpose of this project is to define the regions of the brain, the neuronal pathways, and the neurotransmitter systems, which control endocrine, metabolic and autonomic responses to internal stimuli and to various types of stress and the effects of drugs on these systems. Studies on intracerebral injections of various peptides which are selective agonists of different types of opiate receptors have shown that mu-receptors modulate cardiovascular effects. Vasopressin mediation of baroreflexes in the brain and its role in sustaining blood pressure responses to baroreceptor deafferentation were examined,

Objectives: To determine the mechanisms involved in the regulation by the central nervous system of respiratory, metabolic, endocrine and cardiovascular function.

Methods Employed: Surgical techniques for lesioning or microinjection of appropriate pharmacological agents and cerebral ventricles or specific areas of brain, blood pressure, heart rate and changes in plasma catecholamines monitored. Microdissection and microassay of putative neurotransmitters aid identification of specific neuronal systems.

Major Findings: The role of opiate peptides in the regulation of cardiovascular responses to hypotension and control of blood pressure were continued. Intracerebroventricular (icv.) injections of selective opioid agonists were utilized to investigate the role of opiate receptor subtypes in cardiovascular function in awake rats. The μ -agonist (D-Ala², MePhe⁴, Gly⁵-01) enkephalin (DAGO) (1 nmol) caused a prolonged increase in blood pressure and an initial decrease followed by a delayed increase in heart rate. These effects were antagonized by the selective μ -agonist β -flunaltrexamine (β -FNA). A selective δ -agonist (dimeric tetrapeptide enkephalin) (DTE 12) was devoid of cardiovascular effects at 10 nmol, while a κ -agonist (MRZ) caused a pressor response which was not antagonized by β -FNA (Drs. Pfeiffer, Feuerstein, Zerbe, Faden and Kopin).

The mechanisms by which opioids elicit cardiovascular effects were analyzed in detail using microinjections into the anterior hypothalamic area. Low doses of enkephalin produced increases in heart rate and blood pressure. Associate elevations of plasma norepinephrine (NE) and epinephrine (EPI), but not vasopressin, suggested a stimulation of sympathetic-adrenomedullary pathways. Higher doses caused increases in blood pressure but decreases in heart rate. Peripheral vagal blockade with atropine methyl nitrate (ATMN) caused a large sudden rise in heart rate indicating that an increased vagal outflow counteracted the sympathetic activation. Adrenal demedullated rats displayed no tachycardia after anterior hypothalamic injection of low doses of enkephalin, while high doses caused pronounced bradycardia. Additional treatment of demedullated rats with the sympathetic blocker bretylium led to severe hypotension in addition to bradycardia. These data provide evidence that μ -opiate receptors mediate primarily cardiovascular effects of opiates in awake rats. At low doses, a sympathetic adrenomedullary activation occurs, whereas higher doses additionally activate parasympathetic efferents both possibly from anterior hypothalamic sites (Drs. Pfeiffer, Feuerstein, Zerbe, Faden and Kopin).

The acute hypertension and tachycardia which is produced in rats by bilateral section of the nerve fibers entering the Nucleus Tractus Solitarius is accompanied by marked increases in plasma norepinephrine, epinephrine and vasopressin. Similar effects were found in chlorisondamine-treated normal rats and in Brattleboro rats (which cannot form vasopressin). Treatment of Brattleboro rats with chlorisondamine abolished completely the increases in blood pressure and heart rate, indicating that either sympathetic discharge of vasopressin are sufficient to maintain the pressor response to baroreceptor deafferentation, but at least one of these is essential to the response (Drs. Zukowska-Grojec, Bayorh, Zerbe, Palkovits and Kopin). Brattleboro rats are unable to produce vasopressin and are known to be extremely sensitive to hypotensive

shock due to hemorrhage. This sensitivity appears to be due solely to lack of vasopressin since responses to hemorrhage by sympathetic activation and stimulation of the renin-angiotensin system are intact and pithed Brattleboro rats are as sensitive to the pressor agents, i.e., vasopressin, angiotensin, and norepinephrine, as are normal rats.

Vasopressin and catecholamines are present in the Nucleus Tractus Solitarius of rats. In hypertensive rats of the SABRA strain, levels of norepinephrine, epinephrine and vasopressin are higher than in normotensive rats, suggesting that these pressor agents are involved in the response to, or pathogenesis of, this genetic form of hypertension (Drs. Feuerstein, Zerbe, Ben-Ishay, Kopin and Jacobowitz).

Significance to Biomedical Research and the Program of the Institute: The mechanisms involved in the regulation of essential bodily functions to maintain homeostasis or to respond to stress are the basis of survival. Understanding these processes and how they are modified in disease states or by drugs is essential for rational therapy of a wide variety of neuropsychiatric disorders.

Proposed Course: Transfer of the principle investigator to the NINCDS will terminate this project, although various portions will be completed before the other investigators complete their research experience assignments.

Publications:

Zukowska-Grojec, Z., Bayorh, M.A., Zerbe, R.L., Palkovits, M. and Kopin, I.J.: Role of catecholamines and vasopressin in cardiovascular responses to bilateral dorsolateral transection of the medulla oblongata in the rat. *Hypertension* 5: 908-915, 1983.

Zukowska-Grojec, Z., Zerbe, R.L., Jimerson, D., Bayorh, M.A., Palkovits, M. and Kopin, I.J.: Catecholaminergic activity of the baroreceptor areas of the brain in response to bilateral dorsolateral transection of medulla oblongata in rats. *Brain Res*, 1984 (in press).

Pfeiffer, A., Feuerstein, G., Kopin, I.J. and Faden, A.I.: Cardiovascular and respiratory effects of Mu-, Delta- and Kappa-Opiate Agonists Microinjected into the anterior hypothalamic brain area of awake rats. *J. Pharmacol. Exp. Ther.*: 225: 735-741, 1983.

Pfeiffer, A., Feuerstein, G., Zerbe, R.L., Faden, A.I. and Kopin, I.J.: μ -receptors mediate opioid cardiovascular effects at anterior hypothalamic sites through sympatho-adrenomedullary and parasympathetic pathways. *Endocrinology*, 113: 929-938, 1983.

Pfeiffer, A., Feuerstein, G., Faden, A. and Kopin, I.J.: Evidence for an involvement of Mu-, but not delta- or kappa-opiate receptors in sympathetically and parasympathetically mediated cardiovascular responses to opiates upon anterior hypothalamic injection: *Life Sci.*, 31: 1279-1282, 1982.

Pfeiffer, A., Pfeiffer, D.G., Feuerstein, G., Faden, A.I. and Kopin, I.J: An increase in opiate receptor-sites is associated with enhanced cardiovascular depressant, but not respiratory depressant action of morphine. Brain Res. 296: 305-311, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

A01 MH 00271-15 LCS

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Fate of 3-Methoxy-4-Hydroxy-Phenyl Glycol in Primates

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Irwin J. Kopin, Scientific

NINCDS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

THIS PROJECT HAS BEEN COMBINED WITH PROJECT Z01 MH 00403-12-LCS
"Biochemical Indices of Adrenergic Function in Humans and Other
Primates."

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00403-11 LCS

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biochemical Indices of Adrenergic Function in Humans and Other Primates

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Irwin J. Kopin	Scientific Director	NINCDS
Ronald J. Polinsky	Medical Officer (Neurology)	LCS NIMH
David Goldstein	Senior Investigator	NHLBI
Robert T. Brown	Medical Staff Fellow	LCS NIMH
Stanley Burns	Senior Staff Fellow	LCS NIMH
Sanford P. Markey	Chief, Sec. on Analytical Biochemistry	LCS NIMH
David Jimerson	Staff Physician	LCS NIMH

COOPERATING UNITS (if any)

Section on Experimental Therapeutics; NINCDS: Hypertension, Endocrine Branch, NHLBI.

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Medicine

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

7.0

PROFESSIONAL:

5.0

OTHER:

2.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The levels of norepinephrine, epinephrine, and dopamine and their metabolites in various body fluids reflect the activity of the neurons from which these neurotransmitters are released. Urinary catecholamine metabolites provide an index of overall synthesis in the body while the relative proportion of normetanephrine appears to be related to the functional activity. Although plasma levels of norepinephrine reflect the responses of the peripheral sympathetic nervous system it is necessary to consider removal rates of the catecholamine. Cerebrospinal fluid levels of catecholamine metabolites can be used to assess central nervous system metabolism if appropriate corrections are made for the contribution from plasma. These techniques have been applied to studies of catecholamine metabolism in patients with psychiatric disorders and diseases of the autonomic nervous system.

Objectives: To develop methods for assessing adrenergic activity in the brain and peripheral sympatho-adrenal system using assays of catecholamines and their metabolites in body fluids and to apply these methods to the study of disease states and mode of action of therapeutic agents.

Methods Employed: Levels of endogenous catecholamines and their metabolites in cerebrospinal fluid, blood and urine are measured under basal conditions and after evoking a sympathetic response. Isotopically labelled catecholamines or their metabolites are infused intravenously and their levels in the various body fluids examined and related to kinetic parameters. Analyses are performed using liquid scintillation spectrometry, high pressure liquid chromatography and mass spectroscopy.

Major Findings: A simple, reliable method for assay of urinary 3-methoxy-4-hydroxyphenylethylene glycol (MHPG) has been developed using the ethoxy derivative as an internal standard. This method complements others for VMA and normethanephine. Patients with orthostatic hypotension due to brain disease (Multiple System Atrophy, MSA) excrete abnormally low amounts of normetanephine, but almost normal quantities of the deaminated major metabolites, 3-methoxy, 4-hydroxyphenyl glycol (MHPG) and 3-methoxy, 4-hydroxymandelic acid (vanillylmandelic acid, VMA). Patients with Idiopathic Orthostatic Hypotension (IOH) also secreted markedly diminished amounts of normetanephine, but the excretion of VMA and MHPG is also depressed. The disproportionate decrease in normetanephine in patients with MSA is attributed to a lack of stimulation of a relatively intact peripheral sympathetic nervous system. In IOH, all metabolites are diminished because of involvement of the peripheral sympathetic nerves resulting in a proportional decrease in all metabolites.

Infused MHPG labelled with deuterium was used to measure the rates of formation and utilization of free MHPG in plasma. Free MHPG accounted for about two-thirds of the total norepinephrine metabolites in urine, indicating it is the major intermediate in norepinephrine metabolism. In humans as well as monkeys, the deuterated MHPG appears in the CSF (and brain of monkeys), indicating free access of the metabolite from blood to brain.

When a mixture of ^3H - ℓ and ^{14}C - d -norepinephrine is infused with ^3H -isoproterenol, there is a slower decline of plasma ^3H -isoproterenol than of the d - or ℓ -norepinephrine reflecting a lack of uptake of isoproterenol into the sympathetic neurons. Desipramine, an antidepressant which inhibits norepinephrine uptake, slows the decline in ^3H isoproterenol which is unaffected by the drug. Similarly in patients with IOH, but not in those with MSA, the rate of decline of labelled norepinephrine in plasma resembles that of isoproterenol, consistent with absence of sympathetic neurons.

CSF obtained at varying intervals after initiation of a constant infusion of deuterated MHPG contains the labelled compound at concentrations which approach those in plasma by 4-8 hours, indicating that there is an exchange of plasma and CSF MHPG (Drs. Ebert and Kopin) This is consistent with the observation that plasma and CSF levels of free MHPG are highly correlated. CSF levels are always higher than those in plasma, even when large amounts of the catecholamine metabolite are derived from the tumor of the adrenal medulla. This

is explained by a plasma and CSF two-compartment system with similar rate constants for entry into and exit from the CSF compartment. MHPG formed, but not metabolized, in the central nervous system maintains CSF levels of MHPG at a constant increment over those in plasma. Estimation of this increment provides the best available index of formation of MHPG in the central nervous system (Drs. Kopin, Polinsky and Jimerson). In monkeys, deuterated MHPG enters brain from plasma and the exchange of plasma with brain MHPG explains, at least in part, the covariance of MHPG levels in various areas of brain (Drs. Kopin, Burns and Markey).

MHPG in CSF and in plasma are lowered in both IOH and MSA. Normally between 30 and 50 percent of MHPG in CSF is derived from plasma and the remainder from the central nervous system. In patients with MSA, the proportion of CSF MHPG derived from the central nervous system is diminished, whereas in patients with IOH, the proportion of CSF MHPG derived from peripheral norepinephrine is diminished. This is consistent with a central nervous system deficit in MSA and peripheral sympathetic involvement in IOH.

Significance to Biomedical Research and to the Program of the Institute:

Assessment of adrenergic function by biochemical measurements provides a diagnostic tool and provides the insights into mechanisms of neurological and psychiatric disorders which are necessary to develop rational approaches to therapy. MHPG is a major catecholamine metabolite, particularly in brain and CSF. Its rate of excretion has been claimed (incorrectly) to reflect brain adrenergic activity. The usefulness of MHPG levels and excretion as an index of adrenergic activity has been defined and shown to be of value under specific constraints.

Honors: Dr. Irwin J. Kopin was awarded First Prize for 1983 by the Anna Monika Foundation for work on the disposition and metabolism of MHPG.

Proposed Course: This project will be terminated with transfer of the principal investigator to NINCDS with components continued on Z01 MH 00351-10 LCS.

Publications:

Kopin, I.J., Polinsky, R.J., Oliver, J.A., Oddershede, I.R. and Ebert, M.H.: Urinary catecholamine metabolites as indices of sympathetic neuronal dysfunction in patients with orthostatic hypotension. *J.Clin. Endocrinol. Metab.* 57: 632-637 (1983).

Kopin, I.J.: Catecholamines and the cardiovascular system - recent advances. Plenary lecture 5th International Catecholamine Symposium, Goteborg, Sweden, June 1983 (In press).

Polinsky, R.J., Goldstein, D., Horwitz, D., Keiser, H. and Kopin, I.J.: Plasma catecholamine kinetics in patients with chronic autonomic failure and control subjects. 5th International Catecholamine Symposium, Goteborg, Sweden, June 1983 (In press).

- Kopin, I.J.: Avenues of investigation for the role of catecholamines in anxiety. *Psychopathology* 17: 83-97 (1984).
- Kopin, I.J., Blombery, P., Ebert, M.H., Gordon, E.K., Jimerson, D.C., Markey, S.P. and Polinsky, R.J.: Disposition and metabolism of MHPG-CD₃ in humans: Plasma MHPG as the principal pathway of norepinephrine metabolism and as an important determinant of CSF levels of MHPG. In Usdin, E., Sjoqvist, F. and Bertilsson, L. (Eds.): Frontiers in Biochemical and Pharmacological Research in Depression. New York, Raven Press, 1983, 57-68.
- Polinsky, R.J., Jimerson, D.C. and Kopin, I.J.: Chronic autonomic failure: CSF and Plasma 3-methoxy-4-hydroxyphenylglycol. *Neurology* (in press).
- Polinsky, R.J., Samaras, G.M. and Kopin, I.J.: Sympathetic neural prosthesis for managing orthostatic hypotension. *Lancet*, 901-904, 1983.
- Brown, R.T., Oliver, J., Kirk, K.L. and Kopin, I.J.: Determination of urinary 4-hydroxy-3-methoxyphenylethylene glycol in man by high performance liquid chromatography with electrochemical detection. *Life Sci.*, 34:2313-2318, 1984.
- Solomon, S.L., Wallace, E.M., Ford-Jones, E.L., Baker, W.M., Martone, W.J., Kopin, I.J., Critz, A.D. and Allen, J.R.: Medication errors with inhalant epinephrine mimicking an epidemic of neonatal sepsis. *N. Engl. J. Med.* 310: 166-170, 1984.
- Kopin, I.J., Jimerson, D.C., Markey, S.P., Ebert, M.H. and Polinsky, R.J.: Disposition and metabolism of MHPG in humans: application to studies in depression. *Pharmacopsychiat.* 17:3-8, 1984.
- Martin, P.R., Ebert, M.H., Gordon, E.K., Linnoila, M. and Kopin, I.J.: Effects of clonidine on central and peripheral catecholamine metabolism. *Clin. Pharmacol. Ther.*, 35: 322-327, 1984.
- Martin, P.R., Weingartner, H., Gordon, E.K., Burns, R.S., Linnoila, M., Kopin, I.J. and Ebert, M.H. Central nervous system catecholamine metabolism in Korsakoff's psychosis. *Ann. Neurol.* 15: 184-187, 1984.
- Jimerson, D.C., Rubinow, D.R., Ballenger, J.C., Post, R.M. and Kopin, I.J. Peripheral contribution to cerebrospinal fluid MHPG: studies in depressed patients. *Psychopharmacol. Bull.* 19: 665-668, 1983.
- Goldstein, D.S., Horwitz, D., Keiser, H.R., Polinsky, R.J. and Kopin, I.J. Plasma α -[³H]-norepinephrine, α -[¹⁴C]norepinephrine, and d, α -[³H]isoproterenol. Kinetics in essential hypertension. *J.Clin. Invest.* 72:1748-1758, 1983.
- Jimerson, D.C., Rubinow, D.R., Ballenger, J.C., Post, R.M. and Kopin, I.J. CSF norepinephrine metabolites in depressed patients: New Methodologies. Proceedings of 5th Internl. Catecholamine Symposium, Goteborg, Sweden, 1983 (in press).

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00405-05 LCS

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (90 characters or less. Title must fit on one line between the borders.)

Regulation of Physiological Activity of Aminergic Receptors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
Irwin J. Kopin	Scientific Director	NINCDS
Charles R. Saller	Guest Worker	LCS NIMH
Giora Feuerstein	Guest Worker	LCS NIMH
Zofia Zukowska-Grojec	Visiting Fellow	LCS NIMH
Mohamed Bayorh	Visiting Fellow	LCS NIMH
Robert Zerbe	Senior Staff Fellow	LCS NIMH
Alan I. Faden	Chief, Neurobiology Research Unit	USUHS
David Lozovsky	Guest Worker	LCS NIMH
Chuan-Ching Chang	Special Expert	NINCDS
K.C. Rice	Research Chemist	NIADDK
T.R. Burke	Sr. Staff Fellow	NIADDK
David Jimerson	Chief, Section on Exp. Therapeutics	LCS NIMH

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Medicine

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

3.0

PROFESSIONAL:

2.5

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The objectives of this project are to assess changes in properties (number, affinity) of receptors in response to alterations in the environment, drug administration, stress or hormones, and to develop methods for assessing receptor function in intact animals, in vivo. Studies on the role of prostaglandins in regulation of blood pressure implicated in the pathogenesis of the hypertension in experimental animals have continued. These studies of responses in intact, awake animals provide a necessary complement to studies in pithed rats (Project Z01 MH 00401).

Objectives: Determining the mechanisms by which receptors are activated and the events which lead to a response. This year the focus has been on the influence of arachidonic acid metabolite leukotrienes on cardiovascular responses and direct examination of dopamine receptors.

Methods Employed: Blood pressure and heart rate responses are studied in intact animals and pithed rats. Dopamine receptors are studied by examining behavioral effects of drugs known to activate dopamine receptors or by binding of dopamine receptor ligands to brain membranes in vitro.

Major Findings: Recently the leukotrienes have been identified as the major components of slow reacting substance of anaphylaxis (SRS-A). We previously showed that leukotriene D₄ (LTD₄) produces a dual cardiovascular effect in spontaneously hypertensive (SHR) rats which differs from that seen in normotensive rats of the Wistar-Kyoto (WKY) rats from which they were derived. In both SHR and WKY rats LTD₄ evokes a pressor response, but SHR rats are most sensitive to this agent and in SHR but not in WKY rats, the initial pressor response to LTD₄ is followed by a prolonged hypotension and bradycardia which culminates in death. We now found that administration of Thyrotropin Releasing Hormone (TRH) reverses the late hypotension and bradycardia induced by LTD₄ in SHR rats (Drs. Feuerstein, Zukowska-Grojec, Bayorh, Kopin and Faden).

LTD₄ prevents the pressor response to sympathetic stimulation in pithed rats, without altering the stimulation-induced plasma NE increase. The LTD₄ also blocks the pressor action of angiotensin and vasopressin, suggesting that it blocks vascular smooth muscle contraction distal to the receptors for these pressor agents.

The pressor response induced by LTD₄ is attended by a marked increase in vascular resistance in the heart, splanchnic area, muscles and skin without a change in cardiac output. The blood flow to the kidney is not altered - vascular resistance is actually decreased. During the subsequent hypotensive phase, cardiac output is markedly reduced and blood flow to the various agents decreases with severe myocardial ischemia.

Phencyclidine (PCP) is a widely abused psychoactive drug which appears to involve activation of brain dopaminergic systems. PCP administered for the first time diminishes plasma prolactin, but after 30 days of repeated daily doses of PCP, the drug loses this effect, although motor effects persist (Drs. Lozovsky, Saller, Bayorh, Chiueh, Rice, Burke, Kopin). Chronic PCP treatment lowers slightly, but statistically, ³H-spiroperidone binding to dopamine receptors.

In following a course of research on changes in dopaminergic neurotransmission with altered glucose levels, it was found that the increase in dopamine receptors in brains of rats with experimental diabetes can be prevented by co-administration of lithium (Drs. Lozovsky, Saller and Kopin). Chronic treatment with Domperidone, a dopamine antagonist which acts in the pituitary to increase plasma prolactin levels but does not penetrate the blood brain barrier, increases dopamine receptors in the striatum. The effects on brain dopamine receptors must therefore be indirect, possibly via prolactin or other hormones (Drs. Jimerson, Saller, Lozovsky and Kopin).

Significance to Biomedical Research and to the Program of the Institute: Receptor function alteration is a potential factor in disease states and pharmacological responses. The mode of regulation of receptor function must be further understood to determine how changes in receptors occur in disease or during drug action.

Proposed Course: Although investigations of effects of stress, drugs, hormones etc. on various aspects of receptor function (agonist binding, secondary effects at a biochemical level, e.g., cyclic AMP ion transport and net responses) will be continued, this project will be terminated with transfer of the principle investigator to NINCDS.

Publications:

Feuerstein G.L., Zukowska-Grojec, Z., Bayorh, M.A., Kopin, I.J. and Faden, A.I.: Leukotriene D₄-induced shock is reversed by thyrotropin-releasing hormone. Prostaglandins 26:711-723, 1983.

Zukowska-Grojec, Z., Bayorh, M.A., Faden, A.I., Kopin, I.J. and Feuerstein, G.: Pharmacological interventions in the cardiovascular effects of leukotriene D₄ in pithed spontaneously hypertensive rats. In: Piper, P.J. (Ed.): Leukotrienes and Other Lipoxygenase Products. Chichester, England, John Wiley and Sons, 1983, 113-117.

Feuerstein, G., Lozovsky, D., Cohen, L.A., Labro, V.M., Kirk, K.L., Kopin, I.J. and Faden, A.I.: Differential effect of fluorinated analogs of TRH on the cardiovascular system and prolactin release. Neuropeptides, 1984 (in press).

Bayorh, M.A., Lozovsky, D., Rice, K.C., Burke, T.R., Jr. and Kopin, I.J.: Cardiovascular and plasma prolactin responses to stereoisomers of phencyclidine. Pharmacol. Biochem.Behav. 19:365-367, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00153-07 LCS

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Treatment of Obsessional Children and Adolescents with Chlorimipramine

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Judith L. Rapoport, M. D., Chief, Section on Child Psychiatry, LCS, NIMH
Marcus Kruesi, M. D., Clinical Associate, LCS, NIMH
Martine Flament, M. D., Visiting Associate, LCS, NIMH
Dennis L. Murphy, M. D., Chief, CNB, NIMH
Theodore Zahn, Ph.D., Research Psychologist, LPP, NIMH
Wallace Mendelson, M. D., Chief, Unit on Sleep Studies, CPB, NIMH
Paul Fedio, Ph.D., Acting Chief, CN, NINCDS
Martha Denckla, M. D., Chief, Autism and Behavioral Disorders Section, DNB, NINCDS

COOPERATING UNITS (if any)

Unit on Sleep Studies, CPB, NIMH; Laboratory of Psychology and Psychopathology, NIMH; Clinical Neuropharmacology Branch, NIMH; National Institute of Neurological and Communicative Disorders and Stroke

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Child Psychiatry

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

1.50

PROFESSIONAL:

.75

OTHER:

.75

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

Obsessional disorder of childhood is a disabling illness which remains chronic for about 50% of patients. To date, 27 subjects have been studied, the largest sample of children with this disorder studied prospectively.

The purpose of the study is to compare subjects with age-, sex-matched controls on a neuropsychological, psycholinguistic, and familial measures. In addition, a controlled treatment trial with clomipramine is conducted and prospective follow-up of the obsessive compulsive subjects is underway.

Objectives: To examine clinical, familial, physiological (including sleep-EEG), neuropsychological and neuroradiological measures in childhood Obsessive-Compulsive Disorder. In addition, a double blind placebo controlled trial of chlorimipramine is being conducted; drug response is related to the patients' clinical and biological characteristics.

Methods Employed: Patients are sought on a national level because of the rarity of the disorder. Inclusion criteria are Obsessive-Compulsive Disorder as a primary disturbance. Children must have IQ of 85 or above, and be free from unknown neurological disturbance, or other primary psychiatric disorder.

A modification of the Leyton Obsessional Inventory is used to monitor drug effects on Obsessive-Compulsive symptomatology throughout the 12-week clinical trial. Weekly ratings are made by two physicians and ward nurses on the CPRS.

Plasma levels of norepinephrine and of drug, as well as platelet serotonin, are being assayed at baseline and for each drug phase. Sleep EEG is monitored for each drug phase.

Major Findings: Twenty-seven children have entered the protocol to date - 20 males and seven females. The initial impressions of differences in psycholinguistic and neuropsychological test functioning have held up with these larger samples. Specifically, on visual spatial tasks (Milner's Stylus Maze test and Money's Road Map test), subjects perform more poorly than controls. On psycholinguistic tests, subjects appear to adopt less efficient strategies for processing verbal information as evidenced by poorer performances on the Token test, slower tactile naming, and by less right ear advantage on dichotic listening tasks. The dilemma in interpreting this material focuses on whether subtle cognitive strategies may be different between the groups or whether these differences might be evidence of central nervous system dysfunction at a more basic level. Although memory, reaction time and learning do not distinguish the groups, it is possible that the tasks that do distinguish obsessives from controls are more difficult.

Sleep-EEG distinguish subjects from controls, with obsessives having significantly less Delta sleep, and more Stage One sleep. This is of some interest as previous studies with Tourette's patients had shown decreased Delta sleep as the only specific characteristic differentiating Tourette's from controls' sleep EEG patterns. Analysis is underway to see whether subjects who improve on placebo or on drug have parallel changes in their sleep architecture.

Data from the first 19 children to complete the clomipramine-placebo crossover trial indicate that clomipramine is clearly superior to placebo and that for about half of the subjects, this effect is quite marked. However, there are no variables which predict clinical response; baseline ratings of depression have no relationship to improvement on drug. There was no significant relationship found between plasma concentration of clomipramine or desmethylclomipramine and clinical response.

Significance to Mental Health Research: Obsessive-Compulsive Disorder is a rare but extremely disabling condition. About one third of adults with the disorder had their onset during childhood or adolescence. Children with this condition are often very ill and 50% do not respond well to any treatment. Relative to even other rare conditions, such as infantile autism, there has been virtually no research in this area of childhood mental illness.

Proposed Course of Project: A total of 30 patients will be studied. Baseline clinical measures, plasma tricyclic level, and platelet serotonin will be examined in relation to clinical response to active drug and to placebo. A prospective follow-up is planned for all subjects seen in the study. Some patients may eventually exhibit other disorders and we plan to re-evaluate neuropsychiatric and pharmacological data with "pure" diagnostic subgroups based on follow-up studies.

Publications:

Rapoport, J.: Obsessive Compulsive Disorder. In Shaffer, D., Ehrhardt, A., and Greenhill, L. (Eds.): Diagnosis and Treatment in Pediatric Psychiatry. New York, McMillan, in press.

Berg, C., Behar, D., Zahn, T., and Rapoport, J.: Obsessive Compulsive Disorder- An Anxiety Syndrome? In Gittelman, R. (Ed.): Anxiety Disorders. Guilford Press, New York, in press.

Behar, D., Rapoport, J., Berg, C., Denckla, M., Mann, L., Cox, C., Fedio, P., Zahn, T., and Wolfman, M.: Computerized tomography and neuropsychological test measures in adolescents with obsessive compulsive disorder. Am. J. Psychiatry 141:363-369, 1984.

Flament, M., Rapoport, J., and Berg, C.: Childhood Obsessive Compulsive Disorder. In Insel, T. (Ed.): Obsessive Compulsive Disorder. APA Press, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00161-06 LCS

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Behavioral Effects of Dietary Substances in Normal and Hyperactive Children

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Markus Kruesi, M. D., Section on Child Psychiatry, LCS, NIMH
 Mark Cummings, Ph.D., LDP, NIMH
 Marion Yarrow, Ph.D., LDP, NIMH
 Carolyn Zahn-Waxler, M. D., DP, NIMH

COOPERATING UNITS (if any)

Laboratory of Developmental Psychology, NIMH

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Child Psychiatry

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

1.50

PROFESSIONAL:

.75

OTHER:

.75

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The subacute effects of caffeine (10mg/kg/day) were examined in a group of children selected from a school survey for habitual high or low dietary caffeine intake. The two groups differed in measures of autonomic arousal off caffeine (lower arousal for the high caffeine consumers) and in their response to daily caffeine supplement of their diet (high caffeine consumers exhibited fewer adverse side effects).

The effects of glucose, sucrose (1.75gm/lg), aspartame (80mg/kg) and saccharin are being compared in a group of preschool children considered to have adverse behavioral responses to sugar or aspartame.

Objectives: The relationship between habitual caffeine intake and behavioral response to caffeine in grade school children was examined.

Methods Employed: For the caffeine study, a unique feature was the preliminary epidemiological screening of 800 grade school children from three area parochial schools to ask about 24 hour caffeine intake. From these responses, a group of high consumers (intake of 10mg/kg/day or more) and a group of low consumers (matched for age, sex and classroom teacher) were selected for a double blind two week, crossover challenge study with caffeine (10mg/kg/day) or placebo.

For the sugar-aspartame study, the structured playsetting technique worked out by Drs. Cummings, Zahn-Waxler and Yarrow of the Developmental Psychology Laboratory, NIMH, was employed. In this procedure, children and a familiar comparison are videotaped during prearranged situations - playing with toys, watching two adults argue, etc. Prior to each session, children received aspartame (30mg/kg), glucose or sucrose (1.75gm/kg), or saccahrin flavored diet soda.

In addition, parents of the children in the sugar study are rating home behavior following double blind administration of these same substances by the mother during a routine weekend day.

Major Findings: High caffeine consumers differ from low caffeine consumers even after two weeks of abstinence from caffeine. High caffeine consumers tend to have lower arousal level measured by skin conductance response, and to be rated as more restless by their classroom teacher.

On caffeine, low consumers have significantly more adverse effects and are considered more restless on caffeine than on placebo.

Preliminary data from the first few pairs of children who have been challenged with sugars, aspartame and saccahrin indicate no consistent difference in behavior in relation to substance. However, extensive ratings of videotape and considerably more subjects are needed before statistical analysis is appropriate.

Significance to Mental Health Research: There is widespread concern that modern dietary substances may influence the behavior of normal and disturbed children. However, there are practically no controlled clinical trials which have been carried out either with normal or with disturbed children.

The evidence from the caffeine study suggests that children may self select caffeine in their diet in a manner which is appropriate, and that those with adverse effects may actually avoid caffeine intake.

While no adverse effects of sugar were found in a previous study with a grade school sample, the strongest claims for behavioral reactions come from parents of preschool children. In addition, recent interest in violent aggressive individuals who may become more violent in response to sugar challenge makes study of young aggressive children, in this study, more relevant to such public concern.

Proposed Course of Project: Children are being sought with alleged sugar and/or aspartame sensitivity. Both the structured situation at the NIMH and the home setting with parents as raters should evaluate the influence of setting upon dietary effects, if any.

Publications:

Rumsey, J. and Rapoport, J.: Methodological Issues in the Study of Dietary Influence on Behavior in Pediatric Populations. In Wurtman, R. and Wurtman, J. (Ed.): Advances in Nutrition Research. New York, Raven Press, 6, 1983.

Rapoport, J.: Methodology for assessing effects of dietary substance in grade school children. J. Psychiatr. Res. 17:187-191, 1982-83.

Behar, D., Rapoport, J., Berg, C., Adams, A., and Cornblath, M.: Sugar challenge testing with children considered behaviorally "sugar reactive". J. Nutrition and Behavior 1:277-288, 1984.

Rapoport, J., Kruesi, M.: Behavior and Nutrition: A mini review. Contemporary Nutrition 8:1-2, 1983.

Rapoport, J., Berg, C., Ismond, D., Zahn, T., and Neims, A.: Behavioral effects of caffeine in children: Relationship between dietary choice and effects of caffeine challenge. Arch. Gen. Psychiatry, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00162-05 LCS

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Treatment of Hyperactive Children with Desmethylimipramine

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Judith L. Rapoport, M. D., Chief, Section on Child Psychiatry, LCS, NIMH
 Maureen Donnelly, M. D., Clinical Associate, LCS, NIMH
 Alan Zametkin, M. D., Clinical Associate, LCS, NIMH
 William Z. Potter, M. D., Ph.D., Chief, Unit on Clinical Psychopharmacology,
 CPB, NIMH
 Herbert Weingartner, Ph.D., Psychologist, LPP, NIMH
 Markku Linnoila, M. D., Ph.D., LCS, NIAAA

COOPERATING UNITS (if any)

Laboratory of Psychology and Psychopathology, DIRP, NIMH
 Laboratory of Clinical Studies, NIAAA

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Child Psychiatry

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

.65

PROFESSIONAL:

.40

OTHER:

.25

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

A controlled trial of desmethylimipramine (75mg/day) or placebo is almost completed which compares the acute and subacute (3 weeks) clinical effects of this agent in hyperactive children. Preliminary impressions are that desmethylimipramine is less effective than imipramine. Clinical efficacy will be related to plasma tricyclic concentration, and urinary MHPG.

Project Description:

Objectives: As tricyclic antidepressants have been shown to have short-term beneficial effects for hyperactive children, this study is designed to examine these effects more closely. Desmethylimipramine (DMI) acts primarily by blocking reuptake of norepinephrine (NE). By monitoring plasma MHPG, NE, urinary NE, VMA and MHPG and examining clinical effects, changes in NE metabolism and antihyperactive effects could be examined in relation to plasma drug concentration and the effect of drug on NE metabolism.

Methods Employed: DMI or placebo are being tried in a 3-week, double-blind study; a total sample of 30 children is planned. Home and classroom behavior are monitored weekly.

Major Findings: Thirty children have completed the study. DMI has a weak antihyperactive effect; somewhat less striking than that reported for IMI and for amitriptyline at other centers, and considerably less than that of stimulant drugs.

Significance for Mental Health Research: Hyperactivity is a major clinical problem in child psychiatry. Understanding its mechanism may lead to more effective treatment and possible prevention of disorders including alcoholism, sociopathy and schizophrenia.

Projected Course of Project: The project is nearing completion; during the next six months, the code will be broken, data analyzed for relationships among clinical change, urinary changes in NE and MHPG, and plasma concentration of the drug.

Publications:

Rapoport, J., Langer, D., and Ebert, M.: Pilot trial of mianserin treatment of hyperactive boys. In Greenhill, L. (Ed): Biological Aspects of Child Psychiatry. New York, Spectrum Publishers, 1984, pp. 197-210.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00163-05 LCS

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Naturalistic Study of Activity Levels of Hyperactive Children

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Linda Porrino, Ph.D., Guest Worker, Section on Child Psychiatry, LCS, NIMH
 Judith L. Rapoport, M. D., Chief, Section on Child Psychiatry, LCS, NIMH
 Marcus Kruesi, M. D., Clinical Associate, LCS, NIMH
 Thomas Wehr, M. D., Chief, CPB, NIMH

COOPERATING UNITS (if any)

Clinical Psychobiology Branch, DIRP, NIMH

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Child Psychiatry

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

.20

PROFESSIONAL:

.10

OTHER:

.10

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Because of the availability of new technology for measuring 24 hour motor activity, the NIH actometer, a study has been conducted to examine activity levels of hyperactive children and matched controls during a baseline week. Following this, motor activity of the hyperactive group was compared during amphetamine and placebo treatment periods. This is the first study to examine drug effects on motor activity outside of a laboratory setting. Drug effects were examined in relation to measures of structure of classroom and home environment. Actometer data was compared with traditional clinical measures for hyperactive children.

Project Description:

Objectives: Hyperactive boys and matched controls who were in the same school, grade and classes were followed for a baseline week to compare 24 hour activity patterns for the groups. Following this, hyperactive boys were monitored continuously for four weeks; the effects of amphetamine (15 mg) or placebo were compared in a double-blind ABAB design. The point of the study was to see whether hyperactive children were more restless than controls (rather than more inattentive), in what situations, and to examine the effects of stimulant drugs.

Methods Employed: Motor activity levels during school, free play and home activities were compared and related to measures of attention (Continuous Performance Test), and ratings of school and home structure. Children were monitored with the actometer worn on a belt which was worn even when they slept. Weekly appointments were kept at which time parent and teacher behavior ratings, side effects, hourly diaries of weekly activities and attentional measures were obtained.

Major Findings: A total of 12 patient-control pairs were studied. Findings indicate that hyperactive children are significantly more restless than controls even during sleep. Hyperactives are more active during a variety of activities - the group differences were most striking during school but were also significant after school and on weekends. Motor activity differentiated the groups better than did attentional tasks. Drug effects appear biphasic decreasing activity during the day with some increase in activity in the evening. This "rebound" effect may represent altered receptor sensitivity and has not been reported elsewhere in clinical pharmacology.

This is the first clinical diagnostic study to use actometers as part of outpatient observation. They were powerful tools for possible differentiation of patient groups.

Significance to Mental Health Research: Only a naturalistic study such as this can relate laboratory findings to clinically relevant situations. The nature of "hyperactivity" is poorly understood. Since hyperactive children are truly more restless than their peers, it is important to know for what situations this is true. Furthermore, this study provided data on behavioral "rebound" in the evenings following drug, indicating that for many children a multiple dose schedule is indicated.

This study has restored the credibility of the diagnostic label "hyperactivity" which has been removed as the core feature of the syndrome in DSM-III renamed Attention Deficit Disorder. The study also had shed new light on the qualities of the hyperactivity syndrome. For example, the increased motor activity during sleep indicates that some of the syndrome is relatively situation free. However, in comparison to controls hyperactives have particular difficulty in dampening down their activity level during the most structured school activities. Drug treatment does not lower activity below normal level but gives the children the ability to modulate their activity the way the controls can. The pattern of

motoractivity does not suggest an abnormality in biorythms as the "shape" of the 24 hour motor pattern does not differ, only the "level".

Proposed Course of Project: Further studies with different drug dose and schedules are planned, as well as with different diagnostic groups. The specificity of motor restlessness across syndromes has not been demonstrated.

Studies are planned to see if 24 hour motor activity differentiated conduct disordered children from those with primary diagnosis of Hyperactivity. Effects of dietary substances such as sugar and caffeine will be investigated in young conduct disordered and hyperactive boys. These findings will be reported as part of those separate projects (MH 001610-05 and MH 00301-01), however, and this project as a separate endeavor will be terminated.

Publications:

Porrino, L., Rapoport, J., Ismond, D., Sceery, W., Behar, D., and Bunney, W.: A naturalistic assessment of the motor activity in hyperactive boys: I. Comparison with normal controls. Arch. Gen. Psychiatry. 40:681-687, 1983.

Porrino, L., Rapoport, J., Ismond, D., Sceery, W., Behar, D., and Bunney, W.: A naturalistic assessment of the motor activity of hyperactive boys: II. Stimulant drug effects. Arch. Gen. Psychiatry. 40:688-693, 1983.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00165-04 LCS

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biological Markers of Alcoholism

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Judith L. Rapoport, M. D., Chief, Section on Child Psychiatry, LCS, NIMH
 Martine Flament, M. D., Visiting Associate, LCS, NIMH
 Markku Linnoila, M. D., Chief, LCS, DICBR, NIAAA
 Anil Mukherje, M. D., Ph.D., Chief, Section on Molecular and Developmental
 Genetics, PRB, NICHD
 Irwin J. Kopin, M. D., Scientific Director, NINCDS

COOPERATING UNITS (if any)

Clinical Studies Branch, NIAAA
 Pregnancy Research Branch, NICHD
 Office of Scientific Director, NINCDS

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Child Psychiatry

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

1.5

PROFESSIONAL:

0.75

OTHER:

0.75

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The aim of the project was to measure subjective response to ethanol, and putative biological markers for alcoholism in prepubertal boys who are offspring of alcoholics and in matched controls. Markers include: blood and breath acetaldehyde following a test dose of alcohol, and blood alcohol and acetaldehyde dehydrogenase, and transketolase in cell cultures from skin fibroblasts.

Project Description:

Objectives: There is evidence for a genetic factor particularly among male alcoholics. This pilot project compared blood and breath acetaldehyde for "high risk" and control children, as well as subjective responses to a test dose of ethanol.

Methods Employed: Extensive recruitment through area alcohol treatment programs has produced a sample of 11 high risk children and 11 age-matched controls. Clinical screening and skin biopsy were completed. A challenge of alcohol (0.5 ml/kg) followed by four hours of clinical and biological measures was completed.

Behavioral measures included memory test of Parker, et. al., the standing steadiness test for motor coordination, and mood scale of Shuckett and coworkers. All of these measures are sensitive to alcohol effects in adults and all were adapted for use with grade school children.

Blood samples for epinephrine, norepinephrine and cortisol were obtained at half hour intervals throughout the five hour test period.

Major Findings: Blood acetaldehyde, breath acetaldehyde, and breath alcohol reached peak at 30 minutes but did not differ between groups. Clinically, children did not become overly intoxicated in spite of moderate alcohol dose used. Plasma epinephrine increased significantly with alcohol, while plasma cortisol decreased. These physiological measures did not predict behavioral response to ethanol. In contrast, baseline mood state did predict behavior 30 minutes post alcohol ingestion. Children feeling well at baseline tended to become tired and less talkative; children feeling tired or sad at baseline tended to become more lively.

Skin biopsies were cultured in Dr. Mukherje's laboratory, NICHD. Because of a report of an abnormality in the thiamine pyrophosphate (TPP), requiring enzyme to transketolase (K) in some alcoholics, we have measured the apparent Km for binding TPP to TK in skin fibroblasts of the offspring of alcoholics and age/sex-matched controls. The characteristic Km pattern in cells from a particular patient persisted in serial passages in tissue culture. The low risk children had relatively low Km, whereas the high risk children had significantly higher Km. This work is presented by Dr. Mukherje as a possible genetic marker in children at high risk for alcoholism long before their exposure to ethanol.

Significance to Mental Health Research: As the treatment of alcoholism has been relatively unsatisfactory in adult samples, the identification and possible prevention of high risk individuals assumes great significance. Alcoholism in adults is a major public health problem.

Proposed Course of Project: No further studies are planned.

Publications:

Behar, D., Berg, C., Linnoila, M., Wekon, W., and Rapoport, J.: Behavioral and physiological effects of alcohol in high risk and control children. Alcoholism: Clinical and Experimental Research 7:404-410, 1983.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00177-03 LCS

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders)

Treatment of Hyperactive Children with Monoamine Oxidase Inhibitors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Judith L. Rapoport, M. D., Chief, Section on Child Psychiatry, LCS, NIMH
 Alan Zametkin, M. D., Clinical Associate, LCS, NIMH
 Dennis Murphy, M. D., Chief, CNB, NIMH
 Herbert Weingartner, Ph.D., Chief, Unit on Cognitive Studies, LPP, NIMH
 Markku Linnoila, M. D., Ph.D., Chief, CSB, NIAAA
 Farouk Karoum, Ph.D., APB, NIMH

COOPERATING UNITS (if any)

Section on Experimental Therapeutics, LCS, NIMH; Clinical Studies Branch, NIAAA;
 Adult Psychiatry Branch, NIMH; Clinical Neuropharmacology Branch, NIMH;
 Laboratory of Psychology and Psychopathology, NIMH

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Child Psychiatry

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

1.0

PROFESSIONAL:

0.75

OTHER:

0.25

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Fourteen hyperactive boys were treated with up to 15 mg/day of clorgyline, parnate or deprenyl (10 mg/day), selective and non-selective Monoamine Oxidase Inhibitors, or amphetamine (0.5/mg/kg).

The major findings were that both the selective (clorgyline) and nonselective (tranylcypromine) MAOIs were efficacious in reducing restless and inattentive behaviors. Currently a trial of 1-deprenyl in low dose (10 mg/day) and high dose (30 mg/day) is underway. The aim of the study is to elucidate the neurotransmitter mechanisms mediating stimulant drug effects in hyperactivity.

Project Description:

Objectives: MAO inhibitors have been reported effective in adults with childhood history of Attention Deficit Disorder. The purpose of these studies is to evaluate the efficacy of a series of MAOIs for childhood Attention Deficit Disorder with Hyperactivity, as well as to elucidate the possible neurotransmitter mechanisms in amphetamine treatment of hyperactivity.

Methods Employed: Hyperactive boys were treated with clorgyline (up to 15mg/day) or d-amphetamine (0.5mg/kg), using a double blind crossover design modified by a two week placebo washout period in between drug periods. Urinary catecholamines and metabolites, platelet MAO, and plasma 5 HT were measured to see whether these predicted or reflected drug effects.

After 14 subjects completed these first studies, l-deprenyl, an MAO-B inhibitor (10 mg/day) was substituted, and a total of 12 children to date have completed this study.

Major Findings: For the 14 children completing the first studies, (six received clorgyline, eight received tranlycypromine), the MAOIs appear to be almost as effective as d-amphetamine in reducing hyperactive, inattentive behaviors and improving attention span. There were no adverse reactions to either drug. The time course of these effects was comparable to that for d-amphetamine and much more rapid than the antidepressant effects of MAOIs which usually takes two weeks or more to manifest.

Urinary MHPG decreased on both the MAOIs and d-amphetamine but showed no significant relationship with clinical response. Of methodological interest was the persistent significant decrease in urinary MHPG two weeks following d-amphetamine administration. This has particular significance as most studies assume that two weeks drug free period following d-amphetamine is sufficient to be free of physiological effects.

Significance to Mental Health Research: Hyperactivity is a precursor for adult sociopathy, alcoholism and schizophrenia. Studies on the treatment and pathophysiology of hyperactive children have wide implications for preventing and treatment of these major conditions.

Proposed Course of Project: After completion of the low dose (10 mg/day) of l-deprenyl, a second phase study of up to 30 mg/day as tolerated will be conducted. This will permit comparison of MAO-B inhibitors with MAO-A inhibitor (chlorgyline) and a mixed inhibitor (tranlycypromine) for efficacy in the treatment of ADDH. Following this, a treatment trial with fenfluramine will be conducted.

Publications:

Zametkin, A., Rapoport, J., Murphy, D., Linnoila, M., and Ismond, D.: Treatment of childhood attention deficit disorder with hyperactivity with monoamine oxidase inhibitors. Arch. Gen. Psychiatry, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

201 MH 00178-03 LCS

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Brain Structure and Function in Developmental Neuropsychiatric Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Judith M. Rumsey, Ph.D., Staff Fellow, Section on Child Psychiatry, LCS, NIMH
 Connie Duncan-Johnson, Ph.D., Psychologist, LPP, NIMH
 Richard Coppola, Ph.D., Engineer, LPP, NIMH
 Stanley I. Rapoport, M. D., Chief, LN, NIA
 Ronald Zec, Ph.D., Psychologist, APB, NIMH
 Daniel Weinberger, M. D., Chief, APB, NIMH
 Martha B. Denckla, M. D., Neurologist, NINCDS

COOPERATING UNITS (if any)

Laboratory of Psychology and Psychopathology, NIMH; Section on Autism DNB, NINCDS;
 Section on Brain Aging and Dementia, LN, NIA; Clinical Neuropsychiatry and
 Neurobehavioral Section, APB, NIMH

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Child Psychiatry

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

1.75

PROFESSIONAL:

1.0

OTHER:

.75

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Projects completed now include study of auditory brainstem responses in pervasive developmental disorders, a PET-scan FDG study of autism, a clinical follow-up study of autism, a comparative study of psychiatric symptoms in autistic adults, schizophrenics, manics, and normal controls, and a study of conceptual problem-solving in unusually high-functioning autistic adults.

Ongoing projects include a volumetric CT-scan study of autism, a study of CT-scan asymmetries in autism, neuropsychological studies of high-functioning autistic adults, autonomic nervous system studies of autism, and a correlational study of behavioral symptoms in autistic children and adults with widely varying intelligence and language. In addition, we are recruiting more autistic adults for PET-scans in order to achieve a number adequate to apply a correlational analysis to look at functional associations between brain regions.

Normal control data have now been completely collected for all but the autonomic nervous system studies, where data collection is still in progress. Data on the other projects are being scored and statistically analyzed.

New projects include a series of anatomical and physiological studies of learning disabilities in adults and children. Test batteries for use in subject selection, event-related potentials, EEG spectral analyses, and cerebral blood flow are nearly completed. Initial contacts with various school programs have been made to begin work on the recruitment of subjects. Contacts with personnel at Columbia University who are planning to do related epidemiological studies have been made. Initial plans have been made to screen and recruit subjects identified as part of this larger project.

Project Description:

Objectives: These studies have as their goal the identification of neuroanatomical, neurophysiological, and neuropsychological deficits which characterize developmental neuropsychiatric disorders of autism, attention deficit disorders, dyslexia, and dyscalculia. Additional interests are adult outcomes in both autism and dyslexia and the relationships between various patterns of academic deficits, neuropsychological deficits, psychiatric symptoms, and neurophysiological abnormalities. New studies of learning disabilities will attempt to determine both the sensitivity and diagnostic specificity of ERP and EEG spectral analysis and the role of attentional dysfunction in various subtypes of learning and attentional disorders.

Methods: Methods include CT scans (volumetric measurements and linear measurements of asymmetries), FDG PET-scan, EEG spectral analysis combined with topographic mapping, event-related potentials, measurement of regional cerebral blood flow using xenon inhalation, neuropsychological and academic testing, psychiatric interviews, behavioral questionnaires, and supplementary measures.

Major Findings: PET-scan results on ten autistic adults and 15 matched controls confirm initial results showing diffusely elevated rates of glucose use in autism. Localized abnormalities were not identified with this technique given the limits of resolution and the resting state used in this study.

Psychiatric study of autistic men shows that they share symptoms of negative formal thought disorder with schizophrenic and manic adults and additionally share symptoms of affective flattening with schizophrenics. They do not show symptoms of positive thought disorder seen in schizophrenia and manic illness. Other clinical outcome data support these findings as well and have provided more detailed descriptive data on adult outcomes, particularly in high-functioning autistic individuals.

Cognitive studies of high-functioning autistic men show that considerable cognitive deficits remain in most if a majority, though not all, autistic men with average or higher IQs and good language. The relationship of such cognitive deficits to social impairments appears to be complex, however, as correlations between measures of cognitive and social deficits are generally low and nonsignificant.

Significance to Mental Health Research: PET-scan findings continue to suggest the possibility of some diffuse dysfunction that results in a heightened rate of glucose utilization throughout the brain. Similar findings in Down's Syndrome (Schartz, et. al., 1983) suggest that excessive use of glucose may represent a common feature of cerebral dysfunctions without obvious neuropathology which are associated with developmental disorders. These findings also hold implications for the ability of the current PET-technique with its limited resolution to identify physiological abnormalities in disorders without macroscopic anatomical lesions identifiable on CT-scans.

Psychiatric studies support the independence of autism from adult psychoses and lend validating support for current DSM III nomenclature. Follow-up studies support the notion of symptomatic continuity. The identification of cognitive deficits in autism are more prevalent, pervasive, and integral than previously thought. Nevertheless, findings also suggest that multiple and diverse neuropsychological deficits and positive psychiatric symptoms may be associated with final, common, social impairments.

Proposed Course of Project: Completion of testing of normal controls and data analysis are still in progress on several autism studies. Recruitment efforts and development of additional test measures are in progress for the learning disabilities project. We hope to begin testing dyslexic adults sometime during the summer and to begin a screening survey of records of learning disabled children perhaps in the fall in order to identify specific subgroups of children for intensive study.

Publications:

Rumsey, J. M., Grimes, A. M., Pikus, A. M., Duara, R., and Ismond, D. R.: Auditory brainstem responses in pervasive developmental disorders. Biol. Psychiatry, in press.

Rumsey, J. M.: Conceptual problem-solving in highly verbal, nonretarded autistic men. J. Autism Dev. Disord., in press.

Rumsey, J. M., Rapoport, J. L. and Sceery, W. R.: Autistic children as adults: Psychiatric and behavioral outcomes. J. Am. Acad. Child Psychiatry, in press.

Rumsey, J. and Rapoport, J. L.: Assessing behavioral and cognitive effects of diet in pediatric populations. In R. J. Wurtman and J. J. Wurtman (Eds.): Nutrition and the Brain, Volume I, New York, Raven Press, 1983, pp. 101-161.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00301-02 LCS

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Diagnosis in Child Psychiatry

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Judith L. Rapoport, M. D., Chief, Section on Child Psychiatry, LCS, NIMH
 Maureen Donnelly, M. D., Clinical Associate, LCS, NIMH
 Alan J. Zametkin, J. D., Clinical Associate, LCS, NIMH
 Mary Beth Aist, Ph.D., Psychologist, Johns Hopkins School of Mental Hygiene
 Eric Taylor, M. D., Senior Registrar, The Maudsley Hospital, London
 Michael Pendergast, M. D., Registrar, The Maudsley Hospital, London
 Michael Rutter, M. D., Professor of Child Psychiatry, The Maudsley Hospital,
 London

COOPERATING UNITS (if any)

Johns Hopkins University, School of Mental Hygiene, Baltimore, Maryland;
 Department of Psychiatry, Maudsley Hospital, London;

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Child Psychiatry

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

.55

PROFESSIONAL:

.40

OTHER:

.15

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A cross national study of the diagnosis of childhood hyperkinesis is underway between the U.S. (NIMH) and the U.K. (The Maudsley Hospital) to understand the basis for the widely discrepant rates of diagnosis between the two countries. In the U. S., hyperkinesis accounts for nearly 50% of child guidance clinic cases, while in the U.K. this diagnosis accounts for less than 3%.

A case history exchange has been completed between the two centers, and a longitudinal follow-up study will utilize a research team's diagnosis using ICD 9 and DSM III to examine predictive validity of the two diagnostic schemes.

Project Description:

Objectives: To examine the differences in diagnostic practice between the U.S. and U.K. particularly with reference to the diagnosis of hyperkinesis. Differences between ICD-9, the current European system, and DSM-III, the current American diagnostic system, will be explored.

Method Employed: Patients referred to the Division of Child Psychiatry of the Maudsley Hospital in London, and to the Section on Child Psychiatry, NIMH are being evaluated in a systematic manner. Initial assessment includes a semistructured videotaped interview, standardized interviews with parents and children, and psychometric testing. A first study, termed a "pilot", study has been carried out.

During the year, a total of 23 U. S. Board Certified child psychiatrists and 21 U. K. child specialists rated 40 cases, 20 with accompanying videotape and 20 with case vignettes alone. After brief training, both groups of physicians rated with both the DSM-III and ICD-9 classification systems. Those data have been scored and put on tapes and cards compatible with both institutions computer systems. We are currently working out the statistical handling of these data as the traditional measure of diagnostic agreement, the kappa statistic, is not appropriate with large numbers of raters.

In addition, the research teams of both hospitals are diagnosing the 40 cases with both systems after more extensive training. Here too, the data has been collected, is being scored and will be analyzed using the kappa statistic.

Major Findings: Preliminary scrutiny of the cases indicate that diagnostic discrepancy between the ICD-9 and DSM-III systems may not be attributable to any one factor. The British system has a rather more inclusive concept of Conduct Disorder; however, it is also clear the children with simple motor restlessness and poor attention are unlikely to be referred for evaluation in the U. K., perhaps because stimulant drugs are rarely employed by British child psychiatrists.

The research teams, however, have little difficulty in mastering each other's system. For the research teams, there are some cases which are particularly well diagnosed with ICD-9 while some others, such as cases with conduct disorder, hyperactivity and developmental delay, are difficult to diagnose with ICD-9 because of the rather rigid syndromal classification pattern.

Significance to Mental Health Research: It is of considerable interest to understand the wide variation in rates for childhood hyperactivity across different countries. While it is possible that different genetics, social structure or even environmental toxins might be responsible for such differences, it is essential that similar methodology be employed in order for any true differences to even be established.

Proposed Course of Project: Tapes will be rated by local clinicians and by research teams over the following year. Follow-up studies will compare the predictive validity of both systems with both the U. S. and U. K. populations.

Publications: None to date.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00351-10-LCS

PERIOD COVERED

October 1, 1983 - September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Clinical Pharmacology of the Central Nervous System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

David C. Jimerson, Chief, Section on Experimental Therapeutics, LCS, NIMH

COOPERATING UNITS (if any)

Biological Psychiatry Branch, NIMH; Adult Psychiatry Branch, NIMH; Dept. of Psychiatry & Behavioral Science, Univ. of Washington School of Medicine

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Experimental Therapeutics

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

1.5

PROFESSIONAL:

0.5

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided)

The purpose of this study is to develop techniques for studying central nervous system monoamine metabolism in man, and to assess the value of peripheral neurotransmitter measures as indices of central function. Clinical studies assessing the influence of plasma 3-methoxy-4-hydroxyphenylethylene glycol (MHPG) on cerebrospinal fluid (CSF) levels of the metabolite were continued. Development of an assay for o-methylated metabolites of norepinephrine and epinephrine in plasma and CSF was begun.

Other Professional Personnel Engaged on Project

S.P. Markey	Chief, Section on Analytical Biochemistry	LCS, NIMH
M.H. Ebert	Former Section Chief, Section on Experimental Therapeutics	LCS, NIMH
R.M. Post	Chief	BPB, NIMH
R.J. Polinsky	Staff Neurologist	LCS, NIMH
G. Ko	Staff Psychiatrist	APB, NIMH
M. Raskind	Dept. of Psychiatry & Behavioral Science, Univ. of Washington	

Project Description:

Objectives: The purpose of this study is to develop techniques for the comparative assessment of central and peripheral nervous system monoamine metabolism in man. This work includes when necessary the development and validation of new biochemical assay methodologies. Application of these techniques is assessed in studies of patients with a variety of psychiatric, neuropsychiatric and psychosomatic disorders.

Methods: Biochemical methods for assay of endogenous catecholamine metabolites in tissues and body fluids include gas chromatography - mass spectrometry (GCMS) and high pressure liquid chromatography. Radioimmunoassay techniques are used for assay of hormone levels regulated by catecholamine neurotransmitters in vivo. Physiological regulation of neurotransmitter turnover is evaluated in laboratory rodents using brain tissue and in primates using serial sampling of blood, cerebrospinal fluid (CSF), and urine as necessary.

Major Findings: Development of a GCMS assay for normetanephrine and metanephrine in biological tissues and fluids was begun. Initial work showed that acylation of the metabolites resulted in highly efficient extraction from aqueous samples into an organic phase. Subsequent reaction with a silyl reagent yielded stable, volatile, derivatives with favorable characteristics for analysis by GCMS. Present work is focused on improving the detection sensitivity for the procedure and assessing its specificity in plasma and CSF samples.

Study of the influence of plasma MHPG on CSF levels of the metabolite was extended to various psychiatric patient groups. In collaboration with R. Post, study of a small group of depressed patients indicated that they had relatively elevated CSF MHPG levels in spite of apparently normal metabolite levels in plasma. In contrast, a study in collaboration with R. Polinsky showed that reduced levels of CSF MHPG observed in patients with idiopathic orthostatic hypotension resulted almost entirely from the low plasma levels of the metabolite in this population. A project conducted collaboratively with M. Raskind of University of Washington showed that both plasma and CSF levels of norepinephrine and MHPG were elevated in patients with advanced Alzheimers' disease, suggesting combined central and peripheral increases in norepinephrine turnover in these patients. In collaboration with Dr. G. Ko, study of medication free schizophrenic patients revealed increased plasma MHPG levels during episodes of increased psychosis in patients with chronic undifferentiated schizophrenia. This sympathetic response during increased psychosis may be syndrome specific, as paranoid schizophrenic patients did not show this

relationship. Further studies are planned to assess whether elevated plasma MHPG in association with increased psychosis can be localized to central or peripheral noradrenergic turnover.

Significance to Biomedical Research: Assessing the rate of amine metabolite formation in animals and in patients provides information on the rate and location of neurotransmitter turnover. Development of sensitive assays for the o-methylated metabolites of epinephrine and norepinephrine, in conjunction with our present capability of measuring both the deaminated metabolite dihydroxyphenylethylene glycol (DHPG) and MHPG, will allow more specific assessment of the physiological roles of alternative metabolic pathways. Better understanding of the relationship between blood and CSF metabolite levels facilitates the localization of alterations in catecholamine turnover observed in several major psychiatric syndromes.

Proposed Course: Progress in the development of assay methodologies described above will lead to further studies of catecholamine metabolic pathways in laboratory animals, and application to studies of psychiatric patients when drug free and during treatment with neurotransmitter selective medications.

Publications:

Elchisak M.A., Cosgrove S.E., Ebert M.H., Burns R.S. Distribution of free and conjugated dopamine in monkey brain, peripheral tissues and cerebrospinal fluid determined by high-performance liquid chromatography. Brain Res. 279:171-176, 1983.

Jimerson D.C., Berrettini W.: Cerebrospinal fluid amine metabolite studies in depression: Research update. In Beckmann H. and Riederer P. (eds), Pathochemical Markers of the Psychoses. Berlin: Springer Verlag, in press, 1984.

Raskind M.A., Peskind E.R., Halter J.B., Jimerson D.C.: Norepinephrine and MHPG levels in CSF and plasma in Alzheimer's Disease. Arch. Gen. Psychiatry 41:343-346, 1984.

Jimerson D.C., Rubinow D.R., Ballenger J.C., Post R.M., Kopin I.J.: CSF norepinephrine metabolites in depressed patients: New methodologies. In Usdin E., Carlsson A., Engel J. (eds), Catecholamines. New York: Alan R. Liss, in press, 1984.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00352-09-LCS

PERIOD COVERED

October 1, 1983 - September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pharmacological and Psychometric Studies of Neuropsychiatric Syndromes.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

David C. Jimerson, Chief, Section on Experimental Therapeutics, LCS, NIMH

COOPERATING UNITS (if any)

Laboratory of Psychology and Psychopathology, NIMH; Biological Psychiatry
Branch, NIMH

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Experimental Therapeutics

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS

3.0

PROFESSIONAL:

2.5

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Present clinical studies have focused on the pathophysiology and neuropharmacology of eating disorder syndromes including anorexia nervosa and normal weight bulimia. Serotonin turnover was found to be reduced in bulimic anorectics in comparison to restricting anorectics. During a phase of binge eating, dopamine turnover was reduced in normal weight bulimic patients in comparison to controls. Assessment of α_2 -adrenergic sensitivity to clonidine showed supersensitivity in low weight anorectic patients, with subsequent subsensitivity during the refeeding phase. Studies of the hypothalamic-pituitary-adrenal axis showed blunted corticotropin responses to corticotropin releasing factor (CRF) in low weight anorectic patients. Metabolic studies showed significantly decreased caloric requirements and low plasma insulin levels in normal weight bulimic patients during the binge-free study period.

Other Professional Personnel Engaged on Project:

Z01-MH-00352-09 LCS

W.H. Kaye	Staff Psychiatrist	LPP, NIMH
H. Weingartner	Research Psychologist	LPP, NIMH
L.E. Nee	Clinical Social Worker	LCS, NIMH
S.J. Weiss	Psychologist	LCS, NIMH
H.E. Gwirtsman	Medical Staff Fellow	LCS, NIMH
D.T. George	Medical Staff Fellow	LCS, NIMH
M.H. Ebert	Former Section Chief, Section on Experimental Therapeutics	LCS, NIMH
P.W. Gold	Chief, Unit on Neuroendocrinology	BPB, NIMH
L.M. Bierer	Medical Staff Fellow	BPB, NIMH
R.M. Post	Chief	BPB, NIMH

Project Description:

Objectives: The purpose of this study is to investigate neuropsychiatric syndromes from the perspective of neurotransmitter metabolism, neuroendocrine function, cognitive and psychological function, and pharmacological treatment strategy. Syndromes currently under study include anorexia nervosa and bulimic disorder.

Methods: Methods used in studies of neurotransmitter metabolism include measurement of the parent amine and its major metabolite: e.g., norepinephrine and 3-methoxy-4-hydroxyphenylethylene glycol (MHPG), dopamine and homovanillic acid (HVA), and serotonin and 5-hydroxyindoleacetic acid (5-HIAA). These substances are measured in blood, urine, or cerebrospinal fluid (CSF) by gas chromatography-mass spectrometry or by high performance liquid chromatography. Patient pre-treatment with probenecid prior to CSF studies may allow a better estimation of dopamine and serotonin turnover as reflected in the accumulation of HVA and 5-HIAA.

Methods used in neuroendocrine protocols are established techniques of measuring baseline hormone levels in blood (cortisol, corticotropin (ACTH), prolactin, growth hormone, triiodothyronine, and thyroid stimulating hormone) or urine (free cortisol), as well as responses to hypothalamic releasing factors or to specific neurotransmitter agonists or antagonists. These techniques are valuable in assessing the integrity of feedback regulation in a specific endocrine system, and the sensitivity of neurohormonal and neurotransmitter receptors.

Assessment of neurotransmitter receptor function involves the measurement of physiological responses to relatively selective receptor agonists and antagonists. Physiological responses of interest include altered release of neurotransmitter itself, hypothalamic-pituitary responses, other endocrine responses, and cardiovascular responses (pulse and blood pressure).

Major Findings: Clinical studies of anorexia nervosa and normal weight bulimic disorder have focused on patterns of neurochemical alterations observed at various phases of the illness. The design for studies of patients with anorexia incorporates psychobiologic tests when patients are at low weight, and at short and long-term intervals following weight recovery, all while medication free. Normal weight patients with bulimic disorder are studied initially at a phase in which they have been involved with symptoms of bingeing and vomiting,

and again several weeks later following abstinence from bingeing reinforced by the clinical program.

CSF metabolite levels for anorexic subjects with a history of weight loss by fasting ("restricting anorectics") and levels for subjects with a history of bingeing and purging ("bulimic anorectics") were compared. Serotonin turnover, as reflected in post-probenecid accumulation of the major metabolite 5-hydroxyindole acetic acid in CSF, was significantly reduced in weight-recovered bulimic anorectics in comparison to restricting anorectics. Dopamine and norepinephrine metabolism showed no differences between the two patient groups. The difference in serotonin metabolism is consistent with differences in mood and impulse regulation in these patient groups. In patients with normal weight bulimia studied in the symptomatic phase, central dopamine turnover was reduced, with return to normal levels during abstinence from bingeing. Naturalistic study of bingeing and vomiting behavior in bulimic patients demonstrated progressive increase in the plasma ratio of tryptophan to other neutral amino acids. Further studies will attempt to clarify whether this change in plasma tryptophan alters brain tryptophan and serotonin levels.

To assess norepinephrine receptor function in anorexia nervosa, neurotransmitter and hormonal responses to the α_2 -adrenergic receptor agonist clonidine (2 ug/kg I.V.) were studied. Underweight anorexic patients showed increased responsiveness to the clonidine challenge, while patients studied during the refeeding phase showed marked blunting of the norepinephrine, MHPG, and growth hormone responses to the drug. Since previous studies showed normal plasma norepinephrine concentrations in patients studied at these time points, alterations in pre- and post-synaptic α_2 -receptors may result from changes in neuromodulators such as thyroid hormone levels.

Neuroendocrine studies have revealed abnormalities in both the hypothalamic-pituitary-adrenal and hypothalamic-pituitary-thyroid axes in patients with eating disorders. Preliminary results of infusion studies utilizing corticotropin releasing factor (CRF) - conducted in collaboration with Dr. P. Gold - showed that low weight anorectic patients have a submaximal ACTH response to CRF, but an exaggerated cortisol response. Because of the role of thyroid hormones in modulating thermogenesis, we have assessed thyroid function during low weight episodes and following weight gain in patients with anorexia. The study of low weight patients showed reduced levels of thyroid hormones in comparison to control values, and submaximal, delayed thyroid stimulating hormone responses following infusion of thyrotropin releasing hormone.

Study of cortisol regulation in normal weight patients with bulimic disorder showed normal serum and urinary free cortisol levels. In contrast, these patients manifested an abnormally high incidence of dexamethasone non-suppression when studied early after hospitalization and following an abstinence period of several weeks. Because of evidence from animal studies that catecholamines acting at alpha or beta receptors can elevate plasma corticotropin and cortisol levels, the role of sympathetic nervous system activation in the hypercortisolemia of depression is being studied in collaboration with Drs. L. Bierer and R. Post. Preliminary results of infusions with catecholamine receptor antagonists, taken together with other recent reports, suggest that elevated cortisol levels in depression are not a direct result of the increased catecholamine release observed in some patients.

As a continuation of previous studies of energy metabolism in patients with eating disorders, we assessed average caloric intake and found it to be reduced in patients with normal weight bulimia, in comparison to healthy control subjects. This result supports other evidence that these patients attempt to maintain body weight below a physiological set point. Plasma insulin levels were found to be low following the binge-free abstinent period in bulimic subjects, possibly as a consequence of long-term reductions in caloric intake. Study of serum amylase in bulimic patients showed that blood levels of the enzyme are elevated following binge eating, and that serum amylase levels may be useful for monitoring compliance in treatment programs for bulimia.

Because of evidence for neurobiological differences between anorectic patients with restrictive vs. bulimic eating patterns, we have designed a study to compare psychological traits of restrictor anorexics, bulimic anorexics, normal weight bulimics, and controls. Data collection for this study is nearly completed.

Significance to Biomedical Research: Anorexia nervosa is a disabling neuropsychiatric disorder with substantial morbidity and significant mortality, occurring in 1 of 250 adolescent women. Despite previous attention to psychologic aspects of the disorder, research on the psychobiology of the illness has been relatively neglected. Animal studies have shown that central and peripheral adrenergic systems play a central role in appetite regulation and energy metabolism, and that the activity of noradrenergic systems is in turn under the influence of the HPA, thyroid, and other hormonal systems. Our results demonstrate that specific components of these systems (e.g., α_2 -adrenergic receptors) function abnormally in patients with anorexia. Elucidation of these mechanisms may assist in effective pharmacologic treatment for this disorder.

There has also been increased awareness of the relatively high prevalence of bulimic disorder in young women of normal weight. Our results contribute to evidence that this syndrome may result in part from neurochemical alterations, and that these alterations may have similarities to those postulated to underlie major affective and anxiety disorders.

Proposed Course: Neurotransmitter, neuroendocrine, and metabolite studies in anorexia nervosa will continue with the goal of determining whether these systems play a role in the onset and chronic course of the illness. Further assessment of neurotransmitter and hormonal receptor function is a specific target of these studies. Neurobiological studies of bulimic disorder will continue from the standpoint of comparison to anorexia nervosa, affective illness, and anxiety disorder. Possible new pharmacologic treatment approaches will be evaluated.

Publications:

Gwirtsman HE, Ahles S, Halaris A, DeMet E, Hill M: Therapeutic superiority of maprotiline versus doxepin in geriatric depression. J. Clin. Psychiatry 44:449-453, 1983.

DuBois A, Gross HA, Ebert MH: Gastrinal function in primary anorexia nervosa. In Pirke KM and Ploog D (eds.), The Psychobiology of Anorexia Nervosa. Berlin: Springer Verlag, pp. 87-92, 1984.

Gwirtsman HE, Gerner RH, Sternbach H: Abnormalities in anorexia nervosa of dexamethasone suppression test and urinary MHPG suggest a norepinephrine hypothesis. In Shah S., Donald A. G. (eds.), Psychoneuroendocrine Dysfunction in Psychiatry and Neurological Illnesses: Influence of Psychopharmacological Agents. New York: Plenum Press, pp.129-140, 1984.

Sternbach H., Gwirtsman H., Gerner RH: Biological tests in the diagnosis and treatment of affective disorder. In Shah S., Donald A. G. (eds.), Psychoneuroendocrine Dysfunction in Psychiatric and Neurologic Illnesses: Influence of Psychopharmacological Agents. New York: Plenum Press, pp. 383-398, 1984.

Gwirtsman HE, Roy-Byrne P, Lerner L, Yaeger J: Bulimia in men: Report of three cases with neuroendocrine findings. J. Clin. Psychiatry 45:78-81, 1984.

Baxter L, Edell W, Gerner R, Fairbanks L, Gwirtsman H. Dexamethasone suppression test and axis I diagnosis of inpatients with DSM-III borderline personality disorder. J. Clin. Psychiatry 45: 150-153, 1984.

Ebert MH, Kaye W, Gold PW: Neurotransmitter metabolism in anorexia nervosa. In Pirke KM, and Ploog D. (eds.), The Psychobiology of Anorexia Nervosa. Berlin: Springer Verlag, pp. 58-72, 1984.

Kaye WH, Ebert MH, Raleigh M, Lake CR: Abnormalities in CNS monoamine metabolism in anorexia nervosa. Arch. Gen. Psychiat. 41:350-355, 1984.

Kraemer GW, Ebert MH, Lake CR, McKinney WR: Hypersensitivity to d-amphetamine several years after early social deprivation in rhesus monkeys. Psychopharmacol 82:266-271, 1984.

Caine ED, Ludlow CL, Polinsky RJ, Ebert MH: Provocative drug testing in Tourette Syndrome. J Am Acad Child Psychiatry 23:147-152, 1984.

Gitlin MJ, Gwirtsman HE, Fairbanks L, Sternbach H, Halaris AE, Gerner RH: Dexamethasone suppression test and treatment response. J. Clin. Psychiatry, in press, 1984.

Jimerson, DC: Neurotransmitter hypotheses of depression. Research update. In Lake CR (ed.), Psychiatric Clinics of North America: Clinical Psychopharmacology, Vol I.: Philadelphia: W.B. Saunders, in press, 1984.

Gwirtsman H, Kaye W, Jimerson DC: Pharmacologic treatment of anorexia nervosa and bulimic disorder. In Lake CR (ed.), Psychiatric Clinics of North America: Clinical Psychopharmacology, Vol. II. Philadelphia: W.B. Saunders, in press, 1984.

Jimerson, DC, Cutler NR, Post RM, Rey A, Gold PW, Brown GM, Bunney WE Jr: Neuroendocrine responses to apomorphine in depressed patients and healthy controls. Psychiatry Research, in press, 1984.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00353-02-LCS

PERIOD COVERED

October 1, 1983 - September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.) Biochemical and Pharmacological Studies of Parkinson's Disease and Related Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

R. Stanley Burns, M.D., Senior Staff Neurologist, SET, LCS, NIMH

COOPERATING UNITS (if any)

Sec. on Laboratory Animal Medicine and Care, RSB, NIMH; Office of the Scientific Director, NINCDS; Neuropathology Department of the Armed Forces Institute of Pathology; Lab. of Cerebral Metabolism, NIMH; Sec. on Histopharmacology, LCS,

LAB/BRANCH

Laboratory of Clinical Science

NIMH

SECTION

Section on Experimental Therapeutics

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

1.6

PROFESSIONAL:

0.8

OTHER:

0.80

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The clinical manifestations and biochemical changes in man resulting from exposure to 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) were studied and compared with the findings in patients with idiopathic Parkinson's disease. A unique pattern of change in the concentrations of monoamine metabolites in the cerebrospinal fluid was described. The neuropathological and neurochemical effects of MPTP in the rhesus monkey and beagle dog were studied. The toxic effects of MPTP are selective and result in cell loss of the A9 subgroup of dopaminergic cells in both species indicating that a biochemical difference exists between the A9 and A10 groups.

M.H. Ebert	Former Chief, Sec. on Experimental Therapeutics	LCS NIMH
I.J. Kopin	Scientific Director	NINCDS
J.M. Phillips	Chief, Sec. on Animal Medicine and Care	RSB NIMH
D.M. Jacobowitz	Chief, Sec. on Histopharmacology	LCS NIMH
L. Sokoloff	Chief, Lab. of Cerebral Metabolism, DDBR	LCM NIMH
J. Parisi	Dept. of Neuropathology	AFIP

Project Description:

Objectives: (1) Description of the clinical, biochemical and pharmacological response features of parkinsonism in man induced by N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). (2) Development of the MPTP-treated rhesus monkey and beagle dog as animal models of Parkinson's disease.

Methods: (1) Heroin abusers and chemists with a history of exposure to MPTP were taken off medication, placed on a low monoamine diet, and examined clinically including the measurement of blood pressure (BP) and heart rate (HR). Urine samples were collected for the determination of the 24 hr production rates of catecholamine metabolites using gas chromatography/mass spectrometry (GC/MS) assay methods. Lumbar cerebrospinal fluid (CSF) was obtained for the determination of monoamine metabolite concentrations by GC/MS methods. The clinical response to orally administered L-dopa combined with carbidopa was rated using the Duvoisin scale. (2) The 24 hr excretion rate of catecholamine metabolites in urine and the concentrations of monoamine metabolites in lumbar CSF in the MPTP-treated monkey were determined using GC/MS assay methods. Cardiovascular function (BP and HR) and changes in the level of motor activity were determined with the use of electronic monitoring devices. The effect of L-dopa on the activity level was also measured. MPTP-treated monkeys were sacrificed and the concentrations of monoamines and their metabolites in selected brain regions were measured using the micropunch dissection method coupled with an HPLC assay method. MPTP-treated monkeys were perfused in vivo with formalin and brain sections stained for tissue components including melanin. The pathological changes produced by MPTP were examined by light microscopy. Cerebral glucose utilization in the MPTP-treated monkey were measured using ¹⁴C-2-deoxyglucose and autoradiography. The effect of L-dopa on cerebral glucose utilization was also determined. (3) MPTP was administered intravenously to beagle dogs and the brain examined for pathological changes and dopamine content.

Major Findings: MPTP-induced parkinsonism in man is characterized by hypokinesia, rigidity, resting tremor (early), postural tremor, flexed posture, loss of postural reflexes, mutism and drooling. All of the major clinical features of idiopathic Parkinson's disease are present. Autonomic dysfunction, dementia, psychosis and depression were not present. The level of homovanillic acid (HVA) in the CSF was low (32% of control value), whereas the concentration of 5-hydroxyindolacetic acid was normal and the concentration of 3-methoxy-4-hydroxyphenylethylene glycol was mildly elevated (130%). The urinary excretion rate of HVA and the total metabolites of norepinephrine were normal. The clinical response to L-dopa represented a 65% reversal of the signs and symptoms.

Systemic administration of MPTP to rhesus monkeys results in the development of a neurological syndrome similar to parkinsonism: hypokinesia, rigidity, postural tremor, flexed posture, loss of postural reflexes, drooling and decreased vocalizations. The dopamine (DA) content of the caudate nucleus (12% of control value) and putamen (21%) are decreased and the mole ratio of HVA/DA are increased in animals with mild to moderate motor impairment, whereas the dopamine contents of the nucleus accumbens and olfactory tubercle are not significantly changed. MPTP produces a specific loss of pigmented nerve cells in the pars compacta region of the substantia nigra. The activity level of MPTP-treated monkeys is markedly reduced. L-dopa increases the motor activity to more normal levels and reverses the impairment in swallowing.

MPTP administration to the beagle dog produces nerve cell loss in the substantia nigra region of the midbrain and a marked reduction of the dopamine content of the striatum (less the 5% remaining). MPTP-treated dogs exhibit persistent tremor, 'freezing' episodes and mild bradykinesia which can be reversed by L-dopa.

Significance: The selective toxicity of low doses of MPTP on the A9 subgroup of dopamine producing cells both in the monkey and the dog suggests that a basic biochemical difference exists between the nigrostriatal and other dopamine systems. The selective effect of MPTP on the concentration of HVA in CSF in man is consistent with the findings in animals. MPTP-induced parkinsonism in man represents the neurological syndrome of striatal dopamine deficiency. The findings in MPTP-induced parkinsonism identify which of the clinical features of idiopathic Parkinson's disease are specifically due to the deficiency of dopamine in the striatum.

Proposed course: (1) Now that the MPTP-treated monkey model of Parkinson's disease is well defined, the neurochemical and motor effects of relatively selective D1 and D2 dopamine agonists can be studied. Studies of cholinergic and serotonergic agents are also planned. (2) The kinetics and metabolism of L-dopa will be studied using the MPTP-treated monkey as a model of the damaged 'parkinsonian' brain. (3) Further studies of the biochemical and motor effects of selected pharmacological agents in patients with MPTP-induced parkinsonism are planned.

Publications:

Burns, R.S., Chiueh, C.C., Markey, S.P., Ebert, M.H., Jacobowitz, D.M., and Kopin, I.J.: A primate model of parkinsonism: selective destruction of dopaminergic neurons in the pars compacta of the substantia nigra by N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. Proc. Natl. Acad. Sci. (USA), Vol. 80, pp 4546-4550, July 1983.

LeWitt, P.A., Burns, R.S., Calne, D.B: Lisuride treatment in Parkinson's disease: clinical and pharmacokinetic studies. Adv. Neurol. 37:131-140, 1983.

Burns, R.S., Markey, S.P., Phillips, J.M., and Chiueh, C.C.: The neurotoxicity of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in the monkey and man. The Canadian Journal of Neurological Science, 11 (Suppl. 1): 166-168, 1984.

Burns, R.S., Gopinathan, G., Humpel, M., Dorow, R., Calne, D.B.: Disposition of oral lisuride in Parkinson's disease. Clin. Pharmacol. Ther. 35:548-556, 1984.

Phillips, J.M., Burns, R.S.: The MPTP-treated monkey - an animal model of Parkinson's disease. ILAR News. In press, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00274-10 LCS

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Methods of Ionization in Mass Spectrometry

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

Sanford P. Markey, Chief, Section on Analytical Biochemistry, LCS, NIMH

COOPERATING UNITS (if any)

Biomedical Engineering and Instrumentation Branch, DRS
Department of Pharmacology, George Washington University, Washington, D.C.

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Analytical Biochemistry

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS

1.0

PROFESSIONAL

.5

OTHER

.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided)

An experimental surface ionization tandem mass spectrometer has been constructed and is being used for the analyses of polar organic compounds in complex mixtures of biological origin. Phospholipids, drug metabolites and small peptides have been partially purified and analyzed by liquid surface ionization techniques. Metabolites of MPTP have characterized (see Z01 MH 00279-02) using crude tissue homogenates and tandem mass analysis to separate compounds of interest from background.

Presently, the system is being re-configured to utilize it for spatial analysis of polar organic compounds as an organic ion microprobe. The application of the microwave interface to problems in drug metabolism has been initiated.

PROJECT DESCRIPTION:

Other Professional Personnel Engaged on Project:

Leonid Kelner	Visiting Scientist	BEIB, DRS
Fred P. Abramson	Guest Worker	Professor, Department of Pharmacology, G.W.Univ. Wash. D.C.

Objectives: Improvement of methods in analytical biochemistry requires improvement in instrumentation. Studies were initiated on methods of ionization in mass spectrometry because of the impact of negative chemical ionization on enhancing detection limits, and have been continued to accommodate surface ionization and alternative sample introduction methods.

Methods Employed: Mass spectrometric instrumentation and interfaces are modified as required for each objective.

Major Findings: The collaborative project to design and construct a mass spectrometer suitable for testing ionization methods for use in analytical biochemistry has been continued (see Project No. Z01 RS 10073-04 BEI). The characteristics of this instrument have been determined, and it has been modified to improve ion transmission and mass resolution, and has been applied to the analysis of biological extracts.

MPTP is oxidized to MPP in brain homogenates as shown by direct analysis ethanol extracts from in vitro experiments using deuterated (CD₃-) and non-deuterated MPTP. Clean mass spectra were recorded from the crude homogenates by focusing on the molecular ions; collisionally dissociating the transmitted ions; and mass analyzing the resulting products.

The microwave discharge interface described previously has been transferred to George Washington University where it is being applied to on-going collaborative projects. Metabolites of ¹⁴C-MPTP have been separated on XAD resin and will be analyzed by gc-destructive discharge in order to localize metabolites.

Significance to Biomedical Research: Structure elucidation of unknown compounds in complex mixtures, or the specific detection and quantification of known compounds remain difficult areas of biomedical research. Polar, non-volatile compounds have remained a particular problem. Progress on projects which require these developing analytical methodologies will be substantially speeded, especially in areas of drug metabolism and the determination of unknowns with biological activities,

Proposed Course: The surface ionization mass spectrometer will be tested as an ion microprobe to determine if it can directly quantify organic compounds in tissue slices. The microwave discharge interface will continue to be applied to drug metabolite extracts.

Publications: See Project No. Z01 RS 10073-04 BEI.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00276-05 LCS

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Metabolism of Melatonin

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

Sanford P. Markey, Chief, Section on Analytical Biochemistry, LCS, NIMH

COOPERATING UNITS (if any)

Section on Neuroendrinology, LDN, NICHD Department of Pediatrics, USUHS
Section on Neurocytology, LNNS, NINCDS
Section on Brain Aging and Dementia, LN, NIA

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Analytical Biochemistry

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS

.6

PROFESSIONAL

.1

OTHER

.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided)

The major urinary metabolite of the pineal hormone melatonin, 6-hydroxy-melatonin is being quantified by gas chromatography-negative chemical ionization mass spectrometry. Urinary excretion rates of this metabolite are being used to determine the possible role of the pineal gland in human reproductive biology - i.e., its function during pubertal development and throughout the menstrual cycle. A longitudinal study of melatonin metabolite excretion by young girls is in the fourth year. Each girl has maintained a self-consistent level of excretion, which has not, as yet, correlated with pubertal change. Collaborative studies on transplantation of neonatal rat pineals into pinealectomized rats have begun utilizing the assay of urinary 6-hydroxymelatonin as a measure of pineal function (day and night). Collaborative clinical studies on effects of tricyclic antidepressants and aging are in progress.

Project Description:Other Professional Personnel Engaged on Project:

Merrily A. Poth	Asst. Prof. Pediatrics	USUHS
David C. Klein	Chief, Section on Neuroendocrinology	LDN,NICHD
Milton W. Brightman	Chief, Neurocytology Section	LNNS,NINCDS

Objectives: The role of the pineal gland in human physiology remains undefined. Previously, we developed an assay for the major urinary metabolite of melatonin, the conjugated form of 6-hydroxymelatonin. The urinary assay of this metabolite has been shown to be selective and specific for the pineal hormone, varying diurnally in normal primates, and missing from pinealectomized monkeys or neuronally deficient humans. We have applied the urinary assay to determine whether pineal function is related to pubertal development. This requires daily monitoring of pineal function which can be achieved best by an integrative measure of the urinary metabolite excretion.

The assay of 6-hydroxymelatonin also provides a measure of pineal function useful for pharmacological investigations and various animal studies. Implantation of the pineal gland into pinealectomized rats affords an opportunity to reestablish a circadian oscillating gland in the brain and refine techniques of tissue transplantation which might be applied to other neural tissue transplants. Collaborative clinical studies on the effects of tricyclic antidepressants on humans and aging (esp. Alzheimer's disease) are in progress.

Methods Employed: Urines were collected from human volunteers in 8-hour aliquots to permit some measure of diurnal variation. Conjugated 6-hydroxymelatonin was quantified using gas chromatography-negative chemical ionization mass spectrometry.

Major Findings: The pattern of daily excretion of 6-hydroxymelatonin in young girls studied over a 3-year period has not showed a significant change with pubertal development. The interim findings are suggestive that the pineal gland produces a constant, but individually distinct, amount of melatonin in pre-menarchal girls.

Assays of urines from pinealectomized and pineal transplanted rats have thus far not shown complete regeneration of pineal gland function, although there is a constant but reduced production of 6-hydroxymelatonin (day and night) in transplanted animals.

Melatonin production may provide an index of beta-noradrenergic function as it is affected by various pharmacological agents. For one trial group treated with desipramine, 6-hydroxymelatonin excretion increased by 64%, confirming the hypothesis that an increase in noradrenergic function is involved in antidepressant action.

Significance to Biomedical Research: Studies on the normal physiologic role of melatonin in human biochemistry are lacking. This project is intended to gather baseline data and define the possible role of melatonin in several of its most frequently cited functions. Postulates regarding altered melatonin production as a consequence of altered circadian function and their relation to mental health require these data.

Proposed Course: Continuation of the longitudinal puberty study and collaborative clinical studies to assess the use of 6-hydroxymelatonin as an index of beta-adrenergic function are principle objectives.

Publications:

Poth, M., Higa, S., and Markey, S.P.: The pineal gland and sexual function in man. In Axelrod, J., Frascini, F., and Velo, G.P. (Ed): Endocrine Function of the Pineal Gland. Plenum Press, 509-519, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 00277-05 LCS

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Synthesis of Stable Isotope Labeled Compounds

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

Sanford P. Markey, Chief, Section on Analytical Biochemistry, LCS, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Analytical Biochemistry

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS

.1

PROFESSIONAL

.1

OTHER

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Stable and some radio isotope labeled compounds have been synthesized to support other laboratory projects. Labeled analogues of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine were prepared (see Z01 MH 00279-02 LCS).

Project Description:Other Professional Personnel Engaged on Project:

None

Objectives: The synthesis of labeled compounds is an integral support function to investigations of metabolism and distribution of endogenous and xenobiotic compounds. During the past year, efforts have been focused on labeled variants of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP, Z01 MH 00279-02 LCS) and its metabolites.

Methods Employed: Conventional methods of chemical syntheses employing isotopes have been utilized.

Major Findings: Labeled N-CD₃, N-¹⁴CH₃-MPTP and its fully oxidized metabolite 1-methyl-4-phenylpyridine (MPP⁺) were prepared. Pentadeuterated (phenyl) MPP⁺ was prepared from d₅-bromobenzene as an internal standard for mass spectrometric assays. From this material, variously labeled isotopomers of MPTP were prepared (d₈, d₁₀ by exchange and reduction with borodeuteride). The 4-Cl and 4-F phenyl analogs of MPTP were prepared in gram quantities for animal toxicity testing. Analogues suitable for linkage to proteins are presently being synthesized (1-(4-butyric acid)-4-phenylpyridine).

Significance to Biomedical Research: The availability of labeled compounds is frequently the limiting step in metabolism projects. A program in analytical biochemistry requires continuing synthetic efforts to prepare stable and radioisotope analogues for the timely and efficient solution to metabolism projects.

Proposed Course: Most synthetic efforts will be directed toward yet-to-be elucidated MPTP metabolites. Sufficient quantities to permit toxicity testing in rodents and primates will be prepared. Completion of protein bound MPTP and MPP⁺ analogues is anticipated.

Publications:

None (See Z01 MH 00279-02)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00279-02 LCS

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Pharmacology of Neurotoxins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Sanford P. Markey, Chief, Section on Analytical Biochemistry

COOPERATING UNITS (if any)

Section on Experimental Therapeutics, LCS, NIMH
Section on Histopharmacology, LCS, NIMH
Laboratory of Neurophysiology, NIMH Office of the Director, IRP, NINCDS

LAB BRANCH

Laboratory of Clinical Science

SECTION

Analytical Biochemistry

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS

3.6

PROFESSIONAL

2.8

OTHER

.8

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided)

The mechanism of action of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydro-pyridine (MPTP) is being studied in several animal species. In primates, MPTP produces a persistent parkinsonian syndrome. Neurochemical studies have demonstrated a close correspondence between idiopathic Parkinson's disease in humans and MPTP toxicity. Metabolism of ¹⁴C and ³H MPTP has been studied in four monkeys, sacrificed at times varying from 24 hrs to 20 days. MPTP appears to be metabolized in monkey brain by oxidation to 1-methyl-4-phenylpyridine (MPP) which is trapped intraneuronally. MPP⁺ persists in brain with a half-life of 10 days at micromolar concentrations and may be responsible for MPTP neurotoxicity. In contrast, in the guinea pig and rat, MPTP is rapidly metabolized without accumulation of MPP in brain. The in vitro oxidation of MPTP to MPP has been characterized.

The acute and chronic actions of MPTP in the rat have been characterized. MPTP exhibits acute tryptamine-like effects in the rat, but regardless of the mode of administration, fails to produce a permanent neurotoxicity as observed in the monkey.

The opiate properties of MPTP and the intended synthetic heroin substitute, 1-methyl-4-phenyl-4-propionoxypiperidine (MPPP) have been characterized. It has been shown that MPPP is a potent analgesic which probably masked the slowly developing neurotoxicity of MPTP in drug abusers.

MPP is being studied to determine whether it or its generation is required for expression of neurotoxicity.

Project Description:Other Professional Personnel Engaged on Project:

Chuang Chiueh	Special Expert	OD, IRP, NINCDS
Jan Johannessen	Staff Fellow	SAB, LCS, NIMH
Richard S. Burns	Senior Staff Neurologist	SET, LCS, NIMH
Irwin J. Kopin	Director	IRP, NINCDS
David Jacobowitz	Chief	SH, LCS, NIMH
Miles A. Herkenham	Research Psychologist	LNP, NIMH

Objectives: We have previously described the neurotoxicity of MPTP in man and monkey, both being permanently effected by relatively small amounts (1-2 mg/kg) administered peripherally. Neurochemical and histological examination have confirmed that a parkinsonian syndrome results, with specific lesioning in the caudate and putamen regions, but not in the adjacent nucleus accumbens. The objectives of this project are to determine the mechanism of MPTP neurotoxicity in the primate, and its lack of permanent effects in rodents.

Methods Employed: MPTP toxicity is being studied by qualitative and quantitative observation of animal behavior and locomotion; neurochemical determination of catecholamines and their metabolites in specific brain regions by high pressure liquid chromatography (hplc) with electrochemical detection; determination of the pattern of MPTP distribution, metabolism, and excretion, using radio and stable isotope labelled MPTP (^3H , ^{14}C , ^2H) in several animal species; identification of metabolites unique to the primate and synthesis and pharmacological testing of candidate metabolites. These methods rely upon high specific activity MPTP prepared in this study to characterize by hplc labeled metabolites extracted from physiological fluids or tissues. Autoradiography is being employed to study tissue localization. The structures of isolated metabolites are being determined by mass spectrometry, and will be synthesized and tested *in vivo* to measure their neurotoxicity. Receptor binding studies of MPTP and MPTP metabolites with respect to known or other specific receptors is being pursued.

Major Findings: Metabolism studies of radiolabeled MPTP in several species have shown that in the monkey, MPTP is readily transported to the brain, is concentrated in catecholaminergic neurons, and is oxidized to a pyridinium metabolite (1-methyl-4-phenylpyridine, MPP^+), which appears to be intraneuronally trapped with a terminal phase half-life of 10 days. At 20 days, 0.2% of the total injected dose of MPTP remains in monkey brain, with micromolar concentrations in certain areas demonstrating a high degree of localization. In contrast, in guinea pigs and rats, there is no pattern of MPTP localization in brain at times varying from minutes to several days, and both species rapidly eliminate labeled MPTP. One day after injection, the guinea pig brain retained only 0.1% of the administered dose, the rat brain 0.03%. The pattern of metabolism mirrors the differential neurotoxicity of MPTP in monkeys and rodents.

In rodents, acute effects are severe, but short-lived. In contrast, the persistent effects of MPTP in monkeys are slow to develop - the parkinsonian-like effects appear 3-5 days after a cumulative dose of 1-2 mg/kg, long after the disappearance of MPTP. Oxidation of MPTP to MPP⁺ in the primate provides a facile intracellular trapping mechanism. This trapping is evidenced by the high degree of localization in brain tissue, the absence of MPP⁺ from cerebrospinal fluid, and the ready extractability of MPP⁺ from tissue into water upon homogenization. The toxicity of MPP⁺ intraneuronally trapped may not be directly demonstrable due to its caustic properties and limited ability to cross membranes.

At doses of 5 to 10 mg kg⁻¹, MPTP produces in rats acute immobility, retropulsion, straub tail, piloerection, exophthalmos, salivation and clonic movements of the forepaws. It does not produce analgesia as measured by the tail flick test, nor does it produce permanent motor impairment after chronic or intranigral administration. The acute retropulsion and immobilizing effects can be blocked by methysergide. Administered acutely, MPTP doubles levels of serotonin in the raphe nucleus and substantia nigra. At the same time, levels of dopamine increase in the caudate nucleus and decrease in the substantia nigra. The MPTP-induced decrease in dopamine content of the substantia nigra persists in chronically treated rats, but there is no significant decrease in striatal dopamine. After chronic administration of MPTP, striatal levels of dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were decreased by about 50 percent. Intranigral administrations of MPTP (10 ug daily for 5 days) failed to produce a 6-hydroxydopamine-like lesion in the nigrostriatal system. These results indicate that MPTP in the rat does not cause selective destruction of dopaminergic neurons, but it does produce acute tryptamine-like effects.

The acute and chronic effects of MPTP and 1-methyl-4-(4-chlorophenyl)-1,2,3,6-tetrahydropyridine (Cl-MPTP) on primary cultures of bovine adrenal chromaffin cells demonstrated that MPTP is a cholinergic agonist, intermediate in potency between nicotine and carbachol, whereas Cl-MPTP is a cholinergic antagonist. Exposure of cultured cells to MPTP for up to four days leads to changes in catecholamines similar to that seen with nicotine, but does not lead to cell death as seen in the primate nigrostriatal system.

The initial observation of MPTP neurotoxicity came from drug abusers who were inadvertently exposed to MPTP as a by-product in street preparations of 1-methyl-4-phenyl-4-propionoxyppyridine (MPPP). The opiate properties of these two compounds have been assessed using *in vitro* receptor binding techniques as well as behavioral tests indicative of opiate action, including analgesia, catatonia, respiratory depression, and the loss of righting and corneal reflexes. All opiate activity was found to reside with MPPP, which proved to be potent u-type agonist. The properties of MPPP alone explain repeated abuse of MPTP/MPPP mixtures by heroin addicts.

Significance to Biomedical Research: The MPTP lesioned primate is the best animal model of Parkinson's disease which has been described. The mechanism of action of MPTP may be relevant to cause of idiopathic Parkinson's disease. The blockade of MPTP action may suggest methods to slow the disease progress.

Proposed Course: The apparent toxicity of MPP⁺ will be tested in the primate by (1) inhibition of its formation by MAO inhibitors and (2) promotion of its formation by injection of 1-methyl-4-phenyl-2,3-dihydropyridine.

Publications:

Chiueh, C.C., Burns, R.S., Markey, S.P., Johannessen, J.N., Jacobowitz, D., Ebert, M.H., and Kopin, I.J. : Neurochemical and behavioral effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in rat, guinea pig, and monkey. *Psychopharmacol. Bull.* in press, 1984.

Chiueh, C.C., Markey, S.P., Burns, R.S., Johannessen, J.N., Pert, A. and Kopin, I.J. : Neurochemical and behavioral effects of systemic and intranigral administration of N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in the rat. *Eur. J. Pharmacol.* 100 189-194, 1984.

Johannessen, J.N. and Markey, S.P. : Assessment of the opiate properties of two constituents of a toxic illicit drug mixture. *Drug Alcohol Depend.*, in press, 1984.

Chiueh, C.C., Burns, R.S., Markey, S.P., Jacobowitz, D., Ebert, M.H., and Kopin, I.J. Effects of N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, a cause of an extrapyramidal syndrome in man, on the nigrostriatal dopaminergic system in the rat, guinea pig and monkey. Fifth Catecholamine Symposium Goteborg, Sweden, 98 (Abstr. 74), 1983.

Chiueh, C.C., Markey, S.P., Burns, R.S., Johannessen, J., Jacobowitz, D., Ebert, M.H., and Kopin, I.J. N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, a parkinsonian syndrome causing agent in man and monkey, produces different effects in guinea pig and rat. *Pharmacologist* 25:131 (Abstr. 157), 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00280-02 LCS

PERIOD COVERED

October 1, 1982 through September 30, 1983

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Studies in Morphine Tolerance

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

Jan Johannessen, Staff Fellow, LCS, NIMH

COOPERATING UNITS (if any)

Department of Physiology, Medical College of Virginia, Richmond, VA

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Analytical Biochemistry

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS

0

PROFESSIONAL

0

OTHER

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided)

This project has been discontinued due to the labile nature of behavioral paradigm used to test fractions isolated from the CSF of morphine tolerant rats.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00447-15 LCS

PERIOD COVERED

October 1, 1983, through September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Amine neurotransmitters and metabolites in mental illness

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

William Z. Potter, M.D., Ph.D., Laboratory of Clinical Science, NIMH

COOPERATING UNITS (if any)

Clinical Psychobiology Branch, NIMH; Clinical Neurosciences Branch, NIMH

LAB/BRANCH

Laboratory of Clinical Science

SECTION

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

4.5

PROFESSIONAL:

3.0

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Alterations of amine neurotransmitter systems (norepinephrine (NE), serotonin (5HT) and dopamine (DA)) have been indirectly implicated in the pathophysiology of the major mental illnesses, depression and schizophrenia. We have applied new techniques to study cerebrospinal fluid (CSF), plasma and urine from drug-free patients with affective illness and schizophrenia using more sensitive and comprehensive characterization of the neurotransmitter systems. Careful control of sources of variance continues to produce new lines of evidence consistent with neurotransmitter dysregulation in mental illness:

1. Bipolar vs unipolar depressed patients are consistently found to have lower NE or its metabolite, MHPG, in CSF, plasma and urine, providing strong support for a hypothesis that these subgroups are biochemically distinct.

2. The NE response to physiologic stress is exaggerated in both bipolar and unipolar depression, indicating dysregulation of the system independent of the basal output of NE.

3. We now show high correlations between MHPG in urine, CSF and plasma--evidence against viewing any site as providing differential information.

4. Increases in plasma HVA, a major dopamine metabolite, are associated with poor response to treatment and/or the appearance of psychosis, providing direct biochemical support for a longstanding hypothesis.

5. Initial carefully controlled studies reveal a much clearer circadian rhythm in plasma HVA than MHPG. This is of particular interest since the plasma HVA rhythm appears to coincide with hormonal synthesis of hormones under dopaminergic control.

Other Professional Personnel:

Matthew Rudorfer	Senior Staff Fellow	LCS/NIMH
Robert Golden	Medical Staff Fellow	LCS/NIMH
Michael Sherer	Medical Staff Fellow	LCS/NIMH
Markku Koulu	Visiting Fellow	LCS/NIMH
Markku Linnoila	Chief	LCS/NIAAA
Thomas A. Wehr	Chief	CP/NIMH
David Sack	Senior Staff Fellow	CP/NIMH
David Jimerson	Staff Psychiatrist	LCS/NIMH
David Pickar	Chief, Section on Clinical Studies	CNB/NIMH

Project Description:

The characterization of the functional state of three amine neurotransmitter (NT) systems, NE, 5HT and DA, in depression and other major psychiatric illnesses such as schizophrenia is an ongoing effort in the intramural program. Over a decade of method development and clinical studies has led to the identification of numerous sources of variance which we have only recently been able to control. In many instances, it remains an open question whether appropriate controls are possible.

Simultaneous studies, particularly in depressive illness of neuroendocrine state, peptidergic systems and post-synaptic receptors complement rather than supplant studies of the neurotransmitters themselves and still tend to be conceptualized as either dependent or co-variants of NT function. Even DST escape can be viewed as an HPA abnormality reflecting regulation of the adrenergic/noradrenergic system(s).

Continued expansion of our understanding of the regulation of these NT systems in both healthy volunteers and psychiatric patients seems certain to at least provide tools to subtyping psychiatric illness and predicting response to treatment. Following this classic approach with new techniques still holds the hope of pointing to the underlying pathophysiology of at least some mental illness.

Because of the advances in analytical techniques, the laboratory has been organized into one with central functions.

Methods:

Clinical: Selection of subjects paying particular attention to such issues as age of onset, frequency of recurrence of episodes, and family history is given great emphasis. Whenever feasible, extended (over 1 month) drug-free periods are required before biological samples are obtained—a 3-week period is our current minimum. Patients are also characterized according to length on a low monoamine diet as well as number of days in hospital. This latter parameter is of particular interest since many depressed patients are studied after brief (sometimes only overnight) hospitalization and then transferred to outpatient status.

"Control" subjects are drawn primarily from hospitalized age- and sex-matched individuals who are asked to be on diet. It appears, however, that for comparisons of urine and CSF hospitalization can be a critical variable. Therefore, a comparison of "controls" under different conditions is indicated as an essential component of our design.

Major Findings:

1. We now find that unipolar and bipolar depressed patients can be distinguished more robustly with plasma and CSF measures of NE than with its metabolite, MHPG, in urine, plasma or CSF. Another collaborating group, the Clinical Neurosciences Branch, finds the same in a completely separate group of patients housed on a different ward. In light of the failure of some extramural groups to find unipolar/bipolar differences using different methodological approaches, these new intramural demonstrations assume a special importance.

2. The finding of an elevated NE release on going from a lying to standing position in both unipolar and bipolar depressed patients, dissociated from changes in blood pressure, has been replicated. Interestingly, a collaborating group in the Clinical Neurosciences Branch using a modified technique for obtaining plasma NE finds elevations in the supine position as well as in unipolar, not bipolar, depressions. This may be explained by the practice of the CNB of permitting patients to ambulate in the morning up to within 30 min of drawing the supine blood sample and demonstrates how critical apparently trivial methodological differences can be.

3. Despite previous failures to show meaningful relationships between the NE metabolite, MHPG, in CSF and urine, in our new sets of data with improved control of variance we find the strong positive correlation predicted on theoretical grounds. This coupled with the replication of the strong correlation between plasma and CSF MHPG provides strong evidence that this lipophilic metabolite is in approximate steady-state equilibrium in the three major tissues studied in man and that to the extent MHPG can be used as a measure of NE output, sampling from any compartment provides the same information.

4. Both in our own Section and in collaborative studies with the CNB, increased plasma HVA emerges as a possible biochemical index of deterioration of clinical state accompanied by psychosis. In the CNB studies, during withdrawal from neuroleptics, schizophrenic patients worsen as plasma HVA increases. In our studies, depressed patients worsen and become psychotic as HVA increases following treatment with bupropion. Control studies of HVA in baseline states are under way.

5. Collaborative studies with the CPB and CNB show that normal healthy male volunteers may have a marked circadian pattern of plasma HVA excretion characterized by a peak in the middle of the night and a relatively subtle MHPG rhythm. These findings highlight the possibility that plasma HVA may be a marker of one or more important endogenous rhythms.

Significance to Biomedical Research and to the Program of the Institute:

The major theories about the biological causes of the most prevalent severe psychiatric disorders, depression and schizophrenia, center on monoamine neurotransmitter systems. This project applies sophisticated laboratory assays directly to human studies of monoamine metabolism. Results expand our understanding of the role of norepinephrine in depression and the possible mechanisms of action of antidepressant treatments. New leads suggest that schizophrenia(s) may be subtyped on a biochemical as well as brain imaging basis. The personal and social costs of depression and schizophrenia are great. Insofar as careful clinical research, drawing on basic biochemical techniques, can suggest biological factors in these disorders, specific pharmacologic treatments can be developed and tested in therapeutic trials.

Proposed Course:

We will test the repeated finding of decreased NE output in bipolar depressed patients against other physiologic indices, in particular hydroxymelatonin output and neuroendocrine measures to see if true, selective biochemical measures of bipolar illness are possible.

We shall continue to evaluate what are the "controls" and work out determinants other than production and release of the plasma concentration of transmitters and their metabolites--such as renal clearance. Furthermore, we shall design additional studies to characterize the conversion rates of NE to variance metabolites so as to interpret new findings.

We will continue to collaboratively study schizophrenic patients focusing on the DA system and its interaction with NE. A major focus will be on the possible role of increased plasma HVA as a correlate of psychosis and of patterns of HVA as a circadian marker.

Publications:

- Chang, W.-H., Scheinin, M., Burns, R.S. and Linnoila, M.: Rapid and simple determination of homovanillic acid in plasma using high performance liquid chromatography with electrochemical detection. Acta Pharmacol. et Toxicol., 53: 275-279, 1983.
- Linnoila, M., Ninan, P.T., Scheinin, M., Waters, R.N., Chang, W.-H., Bartko, J. and van Kammen, D.P.: Reliability of norepinephrine and major monoamine metabolite measurements in cerebrospinal fluid of schizophrenic patients. Arch. Gen. Psychiatry, 40:1290-1294, 1983.
- Linnoila, M., Cowdry, R., Lamberg, B. and Rubinow, D.: CSF T₃ levels in patients with affective disorders. Biol. Psychiatry, 18:1489-1492, 1983.
- Potter, W.Z., Ross, R.J. and Zavadil, A.P., III: Norepinephrine in the affective disorders. I. Classic biochemical approaches. In The Catecholamines in Psychiatric and Neurologic Disorders, C.R. Lake and M.G. Ziegler (eds.), Butterworth, Inc., Woburn, MA, in press.
- Linnoila, M., Whorton, A.R., Rubinow, D.R., Cowdry, R.W., Ninan, P.T., Waters, R.N.: Cerebrospinal fluid prostaglandin levels in depressed and schizophrenic patients. Arch. Gen. Psychiatry, 40:405-406, 1983.
- Linnoila, M., MacDonald, E., Reinila, M., Leroy, A., Rubinow, D.R., Goodwin, F.K.: RBC membrane adenosine triphosphatase activities in patients with major affective disorders. Arch. Gen. Psychiatry, 40:1021-1026, 1983.
- Linnoila, M., Karoum, F., Miller, T., Potter, W.Z.: Reliability of urinary monoamine and metabolite output measurements in depressed patients. Am. J. Psychiatry, 140:1055-1057, 1983.
- Linnoila, M., Virkkunen, M., Scheinin, M., Nuutila, A., Rimon, R., Goodwin, F.K.: Low cerebrospinal fluid 5-hydroxyindoleacetic acid concentration differentiates impulse from nonimpulsive violent behavior. Life Sciences, 33:2609-2614, 1983.
- Linnoila, M., Litovitz, G., Scheinin, M., Chang, M-D, Cutler, N.R.: Effects of electroconvulsive treatment on monoamine metabolites, growth hormone and prolactin in plasma. Biol. Psychiat., 19:79-84, 1984.
- Seppala, T., Scheinin, M., Capone, A., Linnoila, M.: Liquid chromatographic assay for CSF catecholamines using electrochemical detection. Acta Pharmacol. et Toxicol., in press.
- Scheinin, M., Seppala, T., Koulu, M., Linnoila, M.: Determination of conjugated dopamine in cerebrospinal fluid from humans and non-human primates with high performance liquid chromatography using electrochemical detection. Acta Pharmacol. et Toxicol., in press.

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Muscettola, G., Potter, W.Z., Pickar, D., Goodwin, F.K.: Urinary MHPG and major affective disorders: A replication and new findings. Arch. Gen. Psychiatry, 41: 337-343, 1984.

Extein, I., Pottash, A.L.C., Gold, M.S., Potter, W.Z.: The effect of increased number of cortisol time products on the sensitivity and specificity of the DST for major depression. Psychopharmacology Bulletin, 19:638-642, 1983.

Potter, W.Z.: Psychotherapeutic drugs and biogenic amines: Current concepts and therapeutic implications. Drugs, 28:127-143, 1984.

Linnoila, M., Karoum, F., Potter, W.Z.: Phenylethylamine and tyramine outputs in patients with affective disorders: Behavioral and biochemical correlates. In Frontiers in Biochemical and Pharmacological Research in Depression, E. Usdin, M. Asberg, L. Bertilsson, and F. Sjoqvist (Eds.), Raven Press, New York, 153-158, 1984.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 01850-07 LCS

PERIOD COVERED

October 1, 1983, through September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Clinical pharmacology of antidepressants

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

William Z. Potter, M.D., Ph.D., Laboratory of Clinical Science, NIMH

COOPERATING UNITS (if any)

Pharmacology-Toxicology Program, NIGMS; Clinical Psychobiology Branch, NIMH;
Division of Special Mental Health Research, NIMH; Cl. Neuropharma. Br., NIMH;
Laboratory of Psychol. and Psychopathol., NIMH; Biol. Psychiat. Br., NIMH

LAB/BRANCH

Laboratory of Clinical Science

SECTION

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

5.5

PROFESSIONAL:

3.5

OTHER:

2.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Antidepressant medications are prescribed to millions of Americans, many of whom receive the drug for months to years. Despite increasingly sophisticated studies in animals and the development of more biochemically specific antidepressants, the therapeutic mechanism of action in man remains unknown. Comparison of biochemical effects in CSF, plasma and urine in the same patients is now feasible with new, efficient high performance liquid chromatography assays, and, when coupled with physiologic, behavioral and neuroendocrine measures, allows for clearer systems interpretations of changes. Findings of particular interest include the following:

1. We previously found that five different antidepressant treatments shared the property of decreasing norepinephrine (NE) turnover; we add to that a similar finding after bupropion in patients of particular interest since:
2. Non-response after the atypical new putative antidepressant bupropion is associated with elevated plasma homovanillic acid, the major dopamine (DA) metabolite, and induction of transient psychosis. These findings support the hypothesis that increased efficiency of the NE distinct from the DA system is a prerequisite for antidepressant effect. Moreover, they support the association of excess stimulation of DA with psychosis.
3. ECT treatment appears to normalize the exaggerated plasma NE response to an orthostatic challenge seen in depressed patients as well as decreasing NE turnover.
4. A rat model for studying neuroendocrine response in man has been developed and implicates interactions of serotonin and DA in prolactin increases after drugs.

Other Professional Personnel:

Matthew Rudorfer	Senior Staff Fellow	LCS/NIMH
Philippe Lesieur	Visiting Fellow	LCS/NIMH
Markku Koulu	Visiting Fellow	LCS/NIMH
Timo Seppala	Visiting Associate	LCS/NIMH
David Pickar	Chief, Section on Clinical Studies	NS/NIMH
Elizabeth Lane	Visiting Fellow	PRAT/NIGMS
Thomas Wehr	Chief, Clinical Research Unit	CP/NIMH
Markku Linnoila	Chief	LCS/NIAAA
Richard J. Wyatt	Chief	DSMHR/NIMH
Dennis L. Murphy	Chief	CN/NIMH
Farouk Karoum	Research Chemist	DSMHR/NIMH
John Nurnberger	Staff Psychiatrist	CG/NIMH
Elliot Gershon	Chief	CG/NIMH
Herbert Weingartner	Psychologist	LPP/NIMH
Judy Rapoport	Chief	CH/NIMH

Project Description:

The major thrust is to understand the effects of major somatic anti-depressant treatments on the monoamine neurotransmitter systems in man. Systematic studies of drug action in normal volunteer controls and depressed patients controlling for pharmacokinetic and pre-drug physiologic variance has permitted demonstration of both predicted and unexpected biochemical alterations following norepinephrine (NE) and serotonin (5HT) uptake inhibition, monoamine-oxidase Type A inhibition, lithium and electro-convulsive therapy (ECT). These treatments share the pharmacodynamic action of reducing NE turnover while increasing NE function.

Comparison of biochemical effects in CSF, plasma and urine in the same patients is now feasible with new, efficient high performance liquid chromatography assays, and, when coupled with physiologic, behavioral and neuroendocrine measures, allows for clearer systems interpretations of changes. State-of-the-art measures of NE, 5HT, DA and their metabolites are made under controlled conditions both cross sectionally in time and longitudinally in order to identify interrelationships, to test assumptions about the regulation of these neurotransmitter systems, and therefore to definitively describe effects of antidepressants as they relate to these neurotransmitter systems.

Methods:

The neurotransmitter systems of patients with either unipolar or bipolar major affective disorder are characterized after at least a 3-week drug-free period and then between the 3rd and 5th week following antidepressant treatment. Certain parameters, such as urinary transmitter and metabolite concentrations, are studied repeatedly following the beginning of each treatment. Parallel studies are performed in healthy volunteers when feasible as described below.

Treatments are ideally administered so as to produce maximal effects on the presumed target biochemical system such as inhibition of NE uptake after desipramine (DMI), of 5HT uptake after citalopram or fluvoxamine, and of MAO-Type A after clorgyline using control of pharmacokinetic variance (blood levels of DMI, citalopram or bupropion) or biochemical indices (MHPG decrease after clorgyline). In the case of lithium and ECT, standard regimens are followed. Novel antidepressants with no clear biochemical specificity such as bupropion and S-adenosylmethionine are also studied.

Studies in college age volunteers housed on the unit are of shorter duration (up to two weeks of active drug) and include DMI, S-adenosylmethionine and lithium. In addition, interactions of these drugs with alcohol on behavioral and physiologic parameters are measured in volunteers but not depressed patients.

Specialized pharmacokinetic and baseline biochemical studies are performed in volunteers age- and sex-matched to our accumulated patient population. These volunteers come to the clinic on the day of the study.

New, improved and efficient high performance liquid chromatography assays are being developed to study NE, 5HT and DA. These are based on the synthesis of appropriate internal standards, utilization of advances in electrochemical detection, and application of automated techniques to the more "routine" analyses. GC-MS has continued to be used for multiple measures in urine.

Findings to date:

1. Consistent with animal studies and our preliminary reports last year, we show selective decreases of norepinephrine (NE) turnover after a wide range of antidepressant treatments. The specific pattern of change reflected in plasma NE varies with each treatment but supports our notion that the overall efficiency of the noradrenergic system is increased following antidepressants.
2. We have additional evidence that at least following 10 days of lithium there are no effects on the NE system, either in terms of urinary NE and its metabolites, plasma NE or plasma MHPG in healthy controls in contradistinction to findings in patients treated with lithium.
3. S-adenosylmethionine (S-AdoMet) in volunteers following 10 days of intravenous administration in doses purported to be antidepressant in patients both reduces the pulse and NE increases observed on standing without altering the blood pressure response, suggesting that this compound, too, increases the efficiency of the NE system.
4. Bupropion, at least in patients, also reduces NE turnover, although unlike tricyclics and lithium it affects the dopamine system in a proportion of patients. Half of the 10 patients whom we have studied show elevated plasma HVA while on bupropion and have either failed to respond or became transiently psychotic. This finding suggests, in accord with preclinical studies, that bupropion is a partial dopamine agonist with the potential of producing psychosis.

5. Preliminary findings from ECT-treated depressed patients are that the baseline exaggerated orthostatic increments in plasma norepinephrine concentration decline towards normal with successful treatment; this change was not seen in a patient who did not respond to ECT. Expansion of this study, incorporating transmitter analysis in other compartments and comparison with drug-treated patients, is under way.

6. Clorgyline, in doses of 5-15 mg/day (one third of those originally used), continues to prove itself a potent antidepressant in unipolar as well as bipolar patients. Its specificity for the noradrenergic system is supported by new studies in rats which show the greatest and most consistent effects on NE in selected brain regions.

7. A new rat model for studying neuroendocrine response allows for paradigms similar to those used in man and has permitted us to show acute effects of the serotonin uptake inhibitor, zimelidine, on prolactin release and an absence of acute effects of the NE uptake inhibitor, desipramine. This pattern is opposite to that observed in man and raises questions concerning the proposed models of neuroendocrine regulation in man based on rat studies.

8. Some studies have continued on the behavioral effects of putative antidepressants in volunteers. A specific memory defect, causing errors of commission in recalling previously learned material, has been identified in healthy volunteers treated with lithium.

Significance to Biomedical Research and to the Program of the Institute:

Understanding of the mechanism(s) of action of antidepressant treatments produces improved therapeutics, new drugs, tools for studying and investigating the underlying pathophysiology of depression and therefore, ultimately, provides the basis for prevention.

From a therapeutic point of view pharmacokinetic studies have been critical to removing problems related to inappropriate dosing. Moreover, the systematic study of biochemically selective (clorgyline) and novel presumably less toxic agents (SAME, bupropion) provides treatments which are effective in many patients who do not respond to standard antidepressants.

Of ultimate importance is the continued finding that changes of the noradrenergic system are always involved in the process of somatic antidepressant treatments. Although simple deficit or excess catecholamine hypotheses of depression do not explain drug action, it seems clear that to understand the mechanism we must understand the role of NE. More and more investigators are "returning" to studies of the NE system in man.

Proposed Course:

1. Complementary studies with newly available selective NE and 5HT uptake inhibitors are planned to test the generalizability of our findings. A collaborative study on the biochemical effects of ECT has been undertaken with Dr. Max Fink to replicate and extend the results found in a small number of inpatients.

2. Detailed investigation of factors controlling the elimination of NE and metabolites from plasma using deuterated intermediates synthesized at the NIH will be performed in volunteers so as to understand the apparent dissociation of results from those seen in urine.

3. Studies will be performed in healthy volunteers age- and sex-matched to patients to assess acute noradrenergic and neuroendocrine responses to intravenous administration of selective NE and serotonin uptake inhibitors.

4. Alcohol interaction studies are being done in collaboration with the new intramural NIAAA program.

5. SAME will be administered to patients to see if it reverses the pre-treatment abnormality of the NE system which we have recently documented (see Z01 MH-00447-15 LCS).

6. Pharmacokinetic studies will focus on possible active metabolites of bupropion and alprazolam to see if these can explain their unexpected effects in man.

Publications

Potter, W.Z., Lane, E.A. and Rudorfer, M.V.: Hydroxy-metabolite concentrations: role of renal clearance. In Clinical Pharmacology in Psychiatry: (Third International Meeting), L.F. Gram, E. Usdin, S.G. Dahl, P. Kragh-Sorensen, F. Sjoqvist and P.L. Morselli (eds.). Macmillan Press, Ltd., London, England, 203-216, 1983.

Linnoila, M., Karoum, F. and Potter, W.Z.: Effects of antidepressant treatments on "whole body" norepinephrine turnover. In Clinical Pharmacology in Psychiatry, (Third International Meeting), E. Usdin, S. Dahl and L.F. Gram (eds.), Macmillan Press, Ltd., Basingstoke, England, 327-338, 1983.

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Linnoila, M., Karoum, F., Rosenthal, N. and Potter, W.Z.: Electroconvulsive treatment and lithium carbonate: Their effects on norepinephrine metabolism in patients with primary major depressions. Arch. Gen. Psychiatry, 40: 677-680, 1983.

Linnoila, M., Johnson, J., Dubyoski, T., Ross, R., Buchsbaum, M., Potter, W.Z. and Weingartner, H.: Effects of amitriptyline, desipramine and zimelidine alone and in combination with ethanol on information processing and memory in healthy volunteers. Acta Psychiatr. Scand., 68: 175-181, 1983

Potter, W.Z., Rudorfer, M.V. and Lane, E.A.: Active metabolites of antidepressants: pharmacodynamics and relevant pharmacokinetics. In Frontiers in Biochemical and Pharmacological Research in Depression, E. Usdin, M. Asberg, L. Bertilsson and F. Sjoqvist (eds.), Raven Press, New York, 373-390, 1984.

Cutler, N.R., Zavadil, A.P. III, Linnoila, M., Scheinin, M., Rudorfer, M., and Potter, W.Z.: Effects of chronic desipramine on plasma norepinephrine concentrations and cardiovascular parameters in elderly depressed women. Biol. Psychiatry 9:549-556, 1984.

Linnoila, M., Miller, T., Bartko, J. and Potter, W.Z.: Effects of five antidepressant treatments on urinary serotonin and 5-hydroxyindoleacetic acid output in depressed patients. Arch. Gen. Psychiatry, 41:688-692, 1984.

Scheinin, M., van Kammen, D.P., Ninan, P.T. and Linnoila, M.: Effect of propranolol on monoamine metabolites in cerebrospinal fluid of patients with chronic schizophrenia. Clin. Pharmacol. Ther. 36:33-39, 1984.

Rudorfer, M.V., Scheinin, M., Karoum, F., Ross, R.J., Potter, W.Z., and Linnoila, M.: Reduction of norepinephrine turnover by serotonergic drug in man. Biol. Psychiatry, 19:179-193, 1984.

Zavadil, A.P. III, Ross, R.J., Calil, H.M., Linnoila, M., Blombery, P., Jimerson, D.C., Kopin, I.J. and Potter, W.Z.: The effect of desmethylimipramine on the metabolism of norepinephrine. Life Sciences 35:1061-1068, 1984.

- Rudorfer, M.V., Lane, E.A., Chang, W.-H., Zhang, M. and Potter, W.Z. Desipramine pharmacokinetics in Chinese and Caucasian volunteers. Br. J. Clin. Pharmacol., 17:433-440, 1984.
- Spiegel, A.M., Rudorfer, M.V., Marx, S.J., Linnoila, M.: The effect of short term lithium administration on suppressibility of parathyroid hormone secretion by calcium in vivo. J. Clin. Endocrinol. Metab., 59:354-357, 1984.
- Ross, R.J., Scheinin, M., Lesieur, P., Hauger, R.L., Rudorfer, M.V., Siever, L.J., Linnoila, M. and Potter, W.Z.: The effect of clorgyline on noradrenergic function. Psychopharmacology, in press.
- Calil, H.M., Lesieur, P., Gold, P.W., Zavadil, A.P.III, Brown, G.M. and Potter, W.Z.: Hormonal responses to zimelidine and desipramine in depressed patients. Psych. Research, in press.
- Rudorfer, M.V. and Potter, W.Z. Metabolism of drugs used in affective disorders. In The Chemotherapy of Affective Disorders: Theory and Practice. G.B. Baker and W.G. Dewhurst (eds.), Croom Helm Ltd., London, in press.
- Linnoila, M., Johnson, J., Dubyoski, K., Buchsbaum, M., Scheinin, M., Kilts, C.: Effects of antidepressants on skilled performance. Br. J. Clin. Pharmacol., in press.
- Cutler, N.R., Haxby, J., Kay, A.D., Narang, K.P., Lesko, L.J., Costa, L.J., Ninos M., Power, M., Linnoila, M., Potter, W.Z., Vanskiver, C.L., Renfrew, J.W., and Moore, A.M.: Evaluation of zimelidine in Alzheimer's disease: pharmacokinetic biochemical and cognitive measures. Annals of Neurology, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00257-08 LDP

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Effects of CNS Treatment on Intellectual Functioning of Children with Leukemia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Howard A. Moss

Guest Worker

LDP NIMH

OTHER: David G. Poplack

Senior Investigator

POB NCI

COOPERATING UNITS (if any)

Various Children's Hospitals throughout the United States
National Cancer Institute

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been terminated.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02144-04 LDP

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of Normal Families and Families with Psychopathology: A Research Paradigm

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI :	Marian Radke-Yarrow	Chief	LDP/NIMH
Other:	Michael Chapman	Research Psychologist	Max-Planck Inst.
	Leon Kuczynski	Visiting Associate	LDP/NIMH
	Carolyn Zahn-Waxler	Research Psychologist	LDP/NIMH
	Barbara Hollenbeck	Social Science Analyst	LDP/NIMH
	Judy Stilwell	Social Science Analyst	LDP/NIMH
	Sarah Friedman	Research Psychologist	LDP/NIMH
	Anne Mayfield	Social Science Analyst	LDP/NIMH

COOPERATING UNITS (if any)

Max Planck Institute, Berlin, West Germany

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20205

TOTAL ~~MAN MONTHS~~ PERSON YEARS

.40

PROFESSIONAL

.35

OTHER

.05

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The objective of this research is to develop a paradigm by which the child-rearing environment and the child's responding in that environment can be observed and recorded in detail. This is accomplished by having mother, father, and children come to the laboratory, an informal homelike apartment, for a series of half-days. Much of the structure and many of the events in these visits are of the participants' own making. Typical rearing functions and requirements are kept intact. Conditions allow and encourage usual routines, behavior, and emotions (eating, playing, learning, disciplining, resting, being happy, angry, upset, etc.). There is also an underlying experimental structure in the sessions. Standard events are introduced as naturally as possible in order to elicit certain classes of response. Families are selected for study based on a psychiatric screening (SADS): Normal families are those in which mother and father are without a psychiatric diagnosis. Maternal depression (bipolar, unipolar and minor depression) defines the clinical groups. The father's diagnosis in this group is allowed to vary; only schizophrenia and antisocial personality are excluded. Mother, 1 1/2- to 2-year-old, and 5- to 8-year-old sibling are the participants in the first series of sessions. In the followup two years later, father is included: also an unfamiliar (4-year-old) peer is present for a limited period. Situations within the sessions arrange participants in various dyads and triads, and in the total family group. All sessions are videotaped. Individual psychiatric assessments and psychological interviews and testing procedures are included in the procedures.

Project Description:

The objective of this research is to develop a paradigm by which the childrearing environment and the child's responding in that environment can be observed and recorded in detail. This is accomplished by having mother, father, and children come to the laboratory, an informal homelike apartment, for a series of half-days. Much of the structure and many of the events in these visits are of the participants' own making. Typical rearing functions and requirements are kept intact. Conditions allow and encourage usual routines, behavior, and emotions (eating, playing, learning, disciplining, resting, being happy, angry, upset, etc.). There is also an underlying experimental structure in the sessions. Standard events (e.g. mother leaves temporarily, mother is made busy, a visitor arrives, child is required to conform to specific requirements) are introduced as naturally as possible in order to elicit certain classes of response. Families are selected for the study based on a psychiatric screening (SADS): Normal families are those in which mother and father are without a psychiatric diagnosis. Maternal depression (bipolar, unipolar and minor depression) defines the clinical groups. The father's diagnosis in this group is allowed to vary; only schizophrenia and antisocial personality are excluded. Mother, 1 1/2- to 2-year-old, and 5- to 8-year-old sibling are the participants in the first series of sessions. In the followup two years later, father is included; also an unfamiliar (4-year-old) peer is present for a limited period. Situations within the sessions arrange participants in various dyads and triads, and in the total family group. All sessions are videotaped.

Further assessments of the parents include a repeated SADS to cover the time interval between entry into the study and follow-up (2 to 3 years later), an interview to determine the presence of mental illness in other members of the immediate family and in first degree relatives, an interview concerning childrearing values, self-assessment of moods, and an account of critical life events during the period of the study. The children receive individual psychiatric assessments and a test of cognitive abilities. Parent and teacher (when relevant) complete the Achenbach Behavior Check List.

There are a number of reasons for developing a sensitive instrument by which to assess the rearing environment in a detailed systematic way and to observe behavior of parent and child in that environment: (a) Understanding of interactions between genetic or constitutional factors and environmental factors necessitates assessments of learning conditions in terms and in ways that identify and measure environmental variables with a level of precision that is not achievable in global ratings, retrospective reports, and brief experimental procedures. (b) A method by which the rearing environment is made accessible for detailed study is crucial for identifying the processes of transmission of adaptive and maladaptive behavioral patterns. (c) Similarly, many social, cognitive, and affective dimensions of early child development can be assessed only or best with behavior samples of substantial duration, with varied content, and in ecologically valid contexts.

This research strategy is directed to two major substantive objectives: (a) to study normal rearing and behavioral developmental processes, and (b) to investigate the nature of impairments in the rearing functioning of depressed mothers and the functioning of their children. Research on the latter objective, of course, requires the normal comparison group. The paradigm is also the source of data for investigating a number of important methodological issues. It makes possible comparisons of findings obtained through the use of different behavior samples and different instruments.

Cognitive, regulatory, and affective dimensions of parental interactions with their children are observed and coded. Child behavior is assessed in each of these domains. Specific research questions regarding comparisons of rearing and child development in families with normal and depressed mothers, interrelations among rearing variables and among child variables, and developmental analyses of children's social and affective behavior are reported in Z01 MH 02152, 02156, 02169, 02170, 02171, 02172, 02175.

Significance to Biomedical Research:

In order to evaluate environmental contributions to the individual's development, sound and specific measures of the environment are needed. Conceptualization and measurement of environment have been seriously deficient in research on genetic-environment interactions and on specific environmental contributions to children's behavior problems and psychiatric disorders. In this study, an effective research method has been developed.

Proposed Course:

The paradigm development has been completed. Approximately 100 families have been seen through all of the assessments of the initial period. Procedures for the follow-up assessments are in place and families are being seen on return visits. One to two years of data collection will be needed. Subsequent reporting of findings utilizing the data from this paradigm will be in the project reports indicated above.

Publications:

Radke-Yarrow, M. and Kuczynski, L.: Conceptions of environment in child-rearing interactions. In Magnusson, D. and Allen, V.L. (Eds.): Human Development: An Interactional Perspective. New York, Academic Press, 1983, pp. 57-74.

Yarrow, M.R., Waxler, C.Z., and Chapman, M.: Children's prosocial dispositions and behavior. In Mussen, P. H. (Ed.): Manual of Child Psychology (Vol. IV, 4th ed.). New York, John Wiley & Sons, 1983, pp. 469-545.

Yarrow, M.R. and Waxler, C.Z.: Roots, motives and patterning in children's prosocial behavior. In Reykowski, J., Karylowski, J., Bar-Tal, D., and Staub, E. (Eds.): The Development and Maintenance of Prosocial Behavior: International Perspectives. New York, Plenum Press, 1984, 81-99.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02146-05

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Etiology of Problem Aggression in Early Childhood

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	E. Mark Cummings	Staff Fellow	LDP NIMH
Other:	Carolyn Zahn-Waxler	Research Psychologist	LDP NIMH
	Ronald J. Iannotti	Research Psychologist	LDP NIMH

COOPERATING UNITS (if any)

NONE

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland

TOTAL PERSON-YEARS PROFESSIONAL:

1.00

.80

OTHER:

.20

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

One problem with remediation programs for problem aggression may be that they are begun too late, after aggressive styles have become highly stable elements of personality, resistant to change. This research attempts to lay the groundwork for earlier prevention and intervention by studying the early origins of highly aggressive behavior, and the factors that contribute to the development of such patterns. Children are studied beginning as early as one year of age, and followed-up three years later. Two main findings have emerged: (1) There is evidence for an aggressive syndrome in young boys, marked by sensitivity to environmental stress, high intensity of aggression, labile emotions, and high stability of aggression over time and in multiple settings. (2) Anger or conflict between adults acts as a powerful instigator of aggression in young children, increasing the incidence and hostility of aggression. Repeated exposure to background anger increases aggressive reactions.

Project Description:

Aggressive, angry, or hostile individuals pose a serious threat to the safety and welfare of others, but efforts at remediation have not proven to be highly successful. One problem may be that aggressive individuals are identified and placed into treatment too late. The present research attempts to lay the groundwork for earlier prevention and remediation by identifying: (a) very early aggressive patterns and the extent to which they are predictive of later problem aggression, and (b) elements of early environments that promote the formation of aggressive patterns.

The procedures are described in detail in earlier reports. In brief, children are studied beginning as early as one year of age, with some children followed-up three years later. Styles or patterns of aggression have been studied in the home, and specific hypotheses regarding the determinants of aggression have been tested in the laboratory.

Two main findings have emerged. (1) There is evidence of an aggressive syndrome in young boys that is characterized by sensitivity to environmental stress, high intensity of aggression, labile emotions, and high stability of aggression both over time and in multiple settings. The findings suggest that high aggression in boys is partly mediated by an underlying temperament or disposition.

(2) Anger or conflict between adults is a powerful instigator of aggression in young children. Children not only show more frequent aggressive behavior, but are also more prone to intense outbursts of aggression after exposure to anger between adults. Aggressive children are most affected; often they are boys, but sometimes girls also show strong aggressive reactions. A finding of clinical interest is that repeated exposure to background anger increases both the incidence and hostility of aggression. This is relevant to the link between marital discord and divorce and conduct disorders in boys, and suggests that anger and conflict in the home play a significant role in the development of children's problem aggression.

The findings of our studies suggest that well-delineated and stable aggressive syndromes may be present in very early childhood, and that, consequently, early childhood may be an optimal time for intervention.

Significance to Biomedical Research:

This research should provide partial answers to the following clinically significant issues: (1) What dispositional and experiential factors are associated with the early development of aggression? (2) What is the prognosis for change among young aggressive children?

Proposed Course:

The follow-up data are currently being obtained. Coding and analyses of data are underway.

Publications:

Cummings, E.M., Zahn-Waxler, C., Radke-Yarrow, M. Developmental changes in children's reactions to anger in the home. Journal of Child Psychology and Psychiatry, 1984, 25, 63-74.

Cummings, E. M., Zahn-Waxler, C., Iannotti, R. J., Hollenbeck, B., and Radke-Yarrow, M. The early organization of individual differences in aggression and altruism. In Zahn-Waxler, C., Cummings, E.M., Iannotti, R. J. (Eds.), Development of Altruism and Aggression, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02148-05 LDP

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Physiologic Jaundice as a Predictor of Behavioral Function in Preterm Infants

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Sarah L. Friedman	Research Psychologist	LDP NIMH
Other:	Carolyn Zahn-Waxler	Research Psychologist	LDP NIMH
	Morris Waxler	Psychologist	FDA DHHS
	Milton W. Werthmann, Jr	Director of Pediatrics	Washington Hosp. Center

COOPERATING UNITS (if any)

Food and Drug Administration
Washington Hospital Center

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland

TOTAL ~~MAN-YEARS~~ PERSON-YEARS PROFESSIONAL:

.25

.21

OTHER:

.04

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Physiologic jaundice occurs when bilirubin, a breakdown product of the red blood cells, is not detoxified at the appropriate rate. Bilirubin levels in preterm infants are often elevated. This can result in damage to various parts of the brain and can consequently affect a range of behaviors. In this study we wished to determine if the subtle impairment in sensory, neurological, and affective function of low risk preterm infants is associated with physiologic jaundice. Two measures of neonatal physiologic jaundice were correlated with 15 behavioral measures, taken from 37 low-risk black preterm infants tested about one month after their jaundice was cleared. The behavioral measures evaluated visual function, motor function, affect and central nervous system integrity. Higher estimates of bilirubin were associated with poorer visual function, quicker cessation of distress in response to social intervention and longer periods of active sleep. The pattern of the results was similar when the effects of health risks other than jaundice were statistically controlled. The results were similar when jaundice was measured by levels of serum bilirubin rather than by estimates of unbound bilirubin. The results support the hypothesis that some aspects of the diverse adverse sequelae of preterm births are associated with damage to the brain by the neurotoxin bilirubin, even in "physiologic jaundice".

Project Description:

I. Rationale and objectives:

Physiologic jaundice occurs when bilirubin, a breakdown product of the red blood cells is not detoxified at the appropriate rate. Bilirubin accumulates in all neonates but for most infants this is not a serious problem because they produce a liver enzyme, glucuronyl transferase, which detoxifies the bilirubin. However, when an infant is born prematurely, its liver is not sufficiently mature to produce adequate quantities of the liver enzyme to detoxify the bilirubin present in the blood stream. Consequently, the bilirubin crosses the blood brain barrier, negatively and irreversibly affecting various brain areas. It is known that bilirubin diffuses in the retina and the superior colliculi and that it damages motor pathways in the basal ganglia, brain stem and cerebellum. Such damage may lead to impaired visual and neurological function in affected infants. It is also known that high levels of bilirubin may produce serious cognitive, affective, sensory and motor deficits. For example, infants who survive severe neonatal jaundice often are afflicted by kernicterus and are mentally retarded. Those with less jaundice are learning disabled. Consequently, it is reasonable to assume that still lesser levels of bilirubin may have more subtle but significant effects in different areas of functioning. In this study we wished to determine if the subtle impairment in the sensory, neurological and affective function of low risk preterm infants is associated with physiologic jaundice.

II. Methods employed:

The subjects were 37 (20 male; 17 female) black preterm infants who were tested between July 1977 and January 1979 when they reached their expected date of birth (40 weeks post conception). The infants were of relatively low medical risk as evidenced by their Apgar scores, weight at birth, stay in the intensive care nursery and a medical risk score. Mean peak serum bilirubin levels (11.8 mg%) and mean peak ratio of serum bilirubin to total protein (2.2) indicate that the infants were exposed to only mild physiological jaundice. Even though these were relatively low levels of bilirubin, 31 of the 37 subjects were nevertheless treated for jaundice by phototherapy. The length of time from the beginning of the first treatment to the end of the last treatment ranged from 19 to 229 hours with a mean of 79.5 hours. The correlation between peak serum bilirubin and length of treatment was $r = 0.70$. The correlation between the peak ratio of serum bilirubin over simultaneous total protein was $r = 0.69$. The number of days from the time therapy was stopped to the time the child was seen for psychological observations was $\bar{X} = 38.77$.

The behavioral performance of the infants was measured in terms of visual function, motor function, affect expression and central nervous system integrity.

III. Major findings:

The peak ratios of bilirubin to total protein, presumed to reflect amounts of potentially available free bilirubin, were correlated with the measures of visual function, of motor function, of affect expression and of central nervous system (CNS) integrity. In the area of visual function, higher ratios were associated with longer response latencies ($r = .33$; $p < .05$) and with longer first fixations

($r = .37$; $p < .05$). To a lesser extent, higher ratios were also associated with longer total fixation times ($r = .30$; $p = .08$). In the area of affect expression, higher ratios were associated with less persistent cries: Crying infants with higher ratios of bilirubin to total protein were easier to sooth, that is, observers invested less effort in calming them down ($r = -.32$; $p = .05$). Sleep organization was taken as a measure of CNS integrity. Higher ratios were associated with longer periods of active sleep ($r = .38$; $p < .05$) (suggesting a relative disturbance in the overall integrity of the CNS). No significant correlations were found between bilirubin/total protein ratios and motor function. To rule out the possibility that risk factors other than physiological jaundice determined the results, the medical risk score, excluding the information about jaundice, was calculated. Bilirubin/total protein ratios were then correlated with the behavioral measures, statistically partialing out the correlation between the modified risk score and the behavioral measures. The results remained statistically significant.

The second measure of physiological jaundice (peak bilirubin levels in the serum) was correlated with the behavioral measures. The correlations were significant for (a) percent first fixations, (b) percent total fixations and (c) drowsiness, ($r = .40$, $p < .05$; $r = .34$, $p = .05$; $r = .33$, $p < .05$, respectively). The measures of intervention soothing and of active sleep were associated with the peak bilirubin level to a somewhat lesser extent than with the presumed availability of free bilirubin ($r = .30$; $p = .06$; $r = .29$; $p = .08$ respectively). Similar results were obtained when the correlation between medical risks (other than jaundice) and the behavioral measures were partialled out from the above correlations.

In this study the relationships between levels of bilirubin in preterms during their neonatal hospitalization and the later behavioral performance of these preterms in areas governed by brain systems known to be affected by bilirubin penetrance were examined. We hypothesized that visual, motor, affective and central nervous system difficulties should be associated with relatively higher levels of physiological jaundice measured a month prior to the behavioral testing. The results show that, as expected, physiological jaundice is related to poorer performance on some of the behavioral measures.

Significance to Biomedical Research:

The findings lead to questions about the specific mechanisms through which bilirubin affects later visual responsiveness and state regulation in preterms (e.g. destruction of brain cells, degradation of myelin sheath, changes in neurotransmitters). Likewise, our findings raise questions about the timing of phototherapy (preventive phototherapy versus responsive phototherapy) in the treatment of young preterm infants.

Proposed course:

The project has been completed. A scientific paper titled "Effects of physiologic jaundice on behavioral function in low risk preterm infants" has been submitted to a pediatric journal.

Publications:

Zahn-Waxler, C., Friedman, S.L., and Cummings, E.M.: Children's emotions and behaviors in response to infants' cries. Child Dev. 54: 1522-1528, 1983.

Friedman, S. L. and Jacobs, B. S.: Wakefulness and visual responsiveness of low medical risk preterms. Early Child Dev. Care 15: 57-68, 1984.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02150-05

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Adjustment to Stress in Early Adolescence: Environmental & Organismic Factors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Editha D. Nottelmann Senior Staff Fellow LDP/NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health

TOTAL ~~WORK YEARS~~ PERSON YEARS PROFESSIONAL:

.45

.40

OTHER:

.05

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The effects of major transitions on children's psychological adjustment are investigated. Children are studied across the year of transition from elementary school to middle school and junior high school, which coincides with the period of transition from childhood to early adolescence. The focus is on children's reports of their self-concept and self-esteem and on reports from their teachers and peers. Analyses examine normative adjustment in a sample of 445 children as well as the psychological profiles of subgroups at risk because of low self-esteem, poor self-concept, isolation, or aggressive tendencies--all factors that are implicated in behavioral disorders in adolescence.

Project Description:

The study examines the co-occurrence of major transitions in the lives of children and their psychological adjustment. A large group of children is studied across the year of transition from elementary to middle and junior high school, which represents significant imposed change: change in schools, change in academic demands, and change from child to adolescent status. The timing of these transitions coincides with the onset of puberty for some of the children; however, physical maturation or pubertal change as an issue is imposed on all children as they enter secondary school. Adjustment and adaptation are examined in the transitional period, because this convergence of change makes it a stressful period of development.

As reported previously, children generally are able to negotiate this transitional period without difficulty. However, subgroups of children who may be at risk were identified as follows:

- (a) Children who were "off time" in physical maturity in their peer group: they had relatively low self-concepts; in particular, girls who were more mature and boys who were less mature saw themselves as relatively less competent in interpersonal and athletic situations, while their teachers rated them as different from their peers only in physical competence.
- (b) Children of short stature: they reported lower self-esteem and academic competence than tall stature children. Their teachers also rated them low on academic competence.
- (c) Children with aggressive tendencies (61 boys and 21 girls), who represent 18% of the sample, were identified by their responses on a projective questionnaire, which indicated that they are likely to bully and pick fights with other children. They were compared with nonaggressive children of similar sex, grade, age, and socioeconomic status. The aggressive children were found to have significantly lower self-esteem and to see themselves as significantly less competent academically than the nonaggressive children. These differences were more pronounced for girls than for boys. Moreover, the aggressive girls also saw themselves as significantly less competent socially than nonaggressive girls. While fewer girls were aggressive, they seemed to have a worse self-image than the aggressive boys. The teacher ratings were similar and confirmed the children's self-assessment.

Significance for Biomedical Research:

The research provides normative data for psychological adjustment in early adolescence, which is an under-researched period of development. It is documenting both incidence and types of marginal adjustment that are likely to be precursors of serious adjustment problems in later adolescence. In addition, the research demonstrates the usefulness of using self-report measures for early detection of adjustment problems.

Proposed Course:

Three manuscripts are in preparation for publication:

Nottelmann, E. D. Competence and self-esteem during transition from childhood to adolescence.

Nottelmann, E. D., & Welsh, C. J. The long and the short of physical stature in early adolescence.

Nottelmann, E. D., & Welsh, C. J. Self-concept and self-esteem correlates of aggression in early adolescence.

Data evaluation is continuing for the preparation of further manuscripts on subgroups who may be at risk, including (a) children who are "off time" in physical maturity, (b) children who have low self-esteem, and (c) children with low social competence.

Publications:

None

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02152-05 LDP

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Discipline and Parental Control in Families with Affective Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI : Leon Kuczynski Visiting Associate LDP/NIMH

Other: Marian Radke-Yarrow Chief LDP/NIMH
Crazyna Kochanska Guest Researcher LDP/NIMH

COOPERATING UNITS (if any)

NONE

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland

TOTAL MAN YEARS

Person Years

PROFESSIONAL:

.85

OTHER:

.05

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The determinants, content, and effects of parental discipline and control practices in families with normal and clinically depressed mothers are investigated. This study, the basic paradigm for which is described in Annual Report MH 02144, is part of a series of investigations assessing the environmental transmission of competent and disordered patterns of child behavior in families with normal and affectively disturbed parents. Impaired parental skills in managing children's behavior have repeatedly been implicated in the etiology of maladaptive patterns of child behavior, such as noncompliance, aggressiveness, and other antisocial behaviors.

Assessments of parent and child behavior are based on detailed observations of parent-child interaction in a naturalistic setting. Among the specific aspects of parental control that are being investigated are the purpose of parental interventions, the quality and timing of mothers' strategies, and their ability to resolve conflicts successfully after initial noncompliance in children. Children's self-control and emotional reactions to parental interventions are also assessed. The influence of social class of family and of seriousness and current status of mothers' affective disorders are examined.

Project Description:

The determinants, contents and effects of parental discipline and control practices in families with normal and clinically depressed mothers are investigated. This study, the basic paradigm for which is described in Annual Report #MH 02144, is part of a series of investigations assessing the environmental transmission of competent and disordered patterns of child behavior in families with affectively disturbed parents. Impaired parental skills in managing children's behavior have repeatedly been implicated in the etiology of maladaptive patterns of child behavior such as noncompliance, aggressiveness, and other antisocial behaviors. Skillful use of control and disciplinary strategies may promote personal and social competence in children.

The use of ineffective forms of discipline and control may be one consequence of parental depression. Several studies suggest that depressed mothers are less involved in the day-to-day control of their children and, when they do intervene, use punitive forms of discipline. However, this research has been global in nature. One purpose of this study is to provide a detailed behavioral assessment of the control practices of depressed and nondepressed mothers and to examine the effects of their practices on children's self-control, compliance, and regulation of emotions. Specific aspects of parental interventions that are being assessed include the timing of the intervention (e.g., whether the mother intervened early to prevent an anticipated, potential misbehavior or late, in reaction to a misbehavior that has already occurred), the issue involved (which behavior the parent attempts to control), parental adaptation of strategies to different kinds of children's misbehaviors, and the outcome of the parent's intervention (whether the parent ultimately succeeds or fails to elicit the child's cooperation). Parental influence strategies are analyzed in terms of their separate verbal, physical and emotional components. The influences of social class of family and of the current status and seriousness of mothers' affective disorder are examined. An important question is whether difficulty in the management of children's behavior is an enduring feature of depressed mothers or whether it consists of transient problems that are confined to the acute stages of the depressive episode.

Significance to Biomedical Research:

Children of depressed parents are at greater risk for psychopathology and behavioral disorders than are children of normal parents. Research on child development has demonstrated that aberrant parental disciplinary practices are important contributors to children's disordered social and emotional development. How depression affects the parent's abilities to function in controlling child behavior is largely unresearched; yet this variable may contribute significantly in creating a pathogenic environment for young children.

Proposed Course:

Data have been collected on 100 families. An instrument for detailed coding of naturalistic childrearing control episodes has been developed and tested. Coding of videotaped data is underway; nine months of coding time is anticipated.

Publications:

Kochanska, G.: Regulatory theory of personality and the development of prosocial behavior. In Staub, E., Bar-Tal, D., Karylowski, J., and Reykowski, J. (Eds.): The Development and Maintenance of Prosocial Behavior. New York, Plenum Press, 1984, pp. 155-176.

Kuczynski, L.: Socialization goals and mother-child interaction: Strategies for long-term and short-term compliance. Dev. Psychol., in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02153-05 LDP

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Maternal Recall of Child's Early Experience

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Penelope K. Trickett	Senior Staff Fellow	LDP NIMH
Other:	Marian Radke-Yarrow	Chief	LDP NIMH
	Carolyn Zahn-Waxler	Research Psychologist	LDP NIMH

COOPERATING UNITS (if any)

NONE

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland

TOTAL PERSON YEARS

.25

PROFESSIONAL:

.20

OTHER:

.05

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Mother's retrospective reports of certain child behaviors and child-rearing techniques and parallel information obtained at an earlier time when the behaviors were current are investigated. Issues of maternal recall of children's early characteristics are of special relevance in etiological questions of psychopathology. Although previous research has demonstrated that in general the correspondence between contemporaneous and retrospective data is low, there is still much to be learned about what determines when mother's recollections are more or less accurate. (1) Are certain kinds of information recollected more accurately than others? (2) How does the mother's current view of the child color her memories? (3) Does depression in the mother affect her recollections? The subjects are mothers of 26-month-old children who are part of a study of etiology of behavior problems. Data collection and coding are completed. Completed analyses indicate mothers are more accurate in recalling their own childrearing behavior than in recalling certain characteristics of their children. Also, depressed mothers are more accurate in recalling the degree of their own emotional reactivity in certain childrearing situations than are non-depressed mothers.

Project Description:

Much of the available knowledge on childrearing techniques and psychopathology in early childhood has been derived from information obtained by interviewing mothers. Many of these interviews have been retrospective, asking the mother to recall certain characteristics of her child or her child-rearing techniques when her child was at a younger age. Other interviews concentrate on the present but still rely on retrospection to the extent that they require the mother to use memories of previous experiences in order to make generalizations about her own or her child's typical behavior. In this study, the relation between mothers' reconstructions of certain child behaviors and child-rearing techniques and parallel information obtained at an earlier time is investigated. Issues of maternal recall of children's early characteristics are of special relevance in etiological questions of psychopathology. Previous research (Yarrow, Campbell, & Burton, 1970) has demonstrated that the correspondence between mothers' recollections and parallel information obtained at an earlier time is often low and that systematic biases in retrospection can occur. However, there is still much to be learned about the factors that determine when mothers' recollections and generalizations are apt to be more or less accurate. For example, are certain kinds of information recollected more accurately than others? How does the mother's current relationship to the child color her memories? How does depression in the mother affect her recollections?

Mothers in the current research are part of a clinical study of etiological factors in the development of behavior problems of children. The baseline data are observational records by mothers who have been trained as observers and have been providing, on a longitudinal basis (10 to 24 months) extensive data on their children, e.g., children's responses to stressful and pleasurable life events, their aggressiveness and noncompliance, and their prosocial behavior. Mothers also reported on their discipline techniques. When each child was 26 months of age, the mother provided retrospective accounts of her child's and her own behavior parallel to that which she recorded when the child was 18 months old. The SADS interview and DSM-III criteria were used to determine psychiatric diagnosis for each mother. Each mother also filled out a standard child-rearing attitudes measure and a mood checklist.

The collection and coding of all data have been completed. Analyses indicate that, in general, mothers are more accurate about recalling their own child-rearing behavior than about recalling characteristics of the child such as temperament, aggressiveness, etc. In recalling childrearing behavior, mothers are more accurate about certain areas than others. For example, the amount of physical punishment and reasoning used by mothers seems more accurately recalled than other types of discipline such as isolation, withdrawal of privileges, or verbal power assertion.

The data are currently being analyzed in terms of presence or absence of depression in the mother. An early finding is that depressed mothers are more accurate in recalling that they didn't react emotionally when their

child witnessed distress in others. Non-depressed mothers were less accurate in recall because they say they did react when in fact they were unlikely to. Analyses are continuing to determine whether this finding is part of a more general pattern or limited only to recall of emotional reactivity.

Significance to Biomedical Research

This study will result in a delineation of both the types of information that are more or less accurately remembered by mothers and the characteristic of the mother's personality, attitudes and values which affect retrospection. Such a delineation will aid the development of subsequent biomedical and psychiatric research methods which rely on maternal retrospection as the method of choice for obtaining necessary information about children's past histories.

Proposed Course

Statistical analyses of the data are underway, a manuscript is in preparation, and the project should be completed within one year.

Publications:

None

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02155-05 LDP

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Children of Depressed and Normal Parents

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Carolyn Zahn-Waxler	Research Psychologist	LDP NIMH
Other:	Marian Radke-Yarrow	Chief	LDP NIMH
	E. Mark Cummings	Senior Staff Fellow	LDP NIMH
	Ronald J. Iannotti	Research Psychologist	LDP NIMH

COOPERATING UNITS (if any)

NONE

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland

TOTAL PERSON-YEARS:

PROFESSIONAL:

OTHER:

Person Years: .85

.75

.10

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Social-emotional competence, coping, and vulnerability are assessed in a longitudinal study of young children with an affectively ill parent. The sample consists of 44 predominantly middle-class families. Children of mothers with unipolar depression and normal mothers are seen at two and at five years of age. They are observed in a laboratory setting in interaction with their mothers, adult strangers, and familiar playmates. The affective environment is varied experimentally to assess children's social-emotional responses. An earlier investigation using similar procedures to study two-year-old children with a bipolar parent indicated significant problems in the quality of interpersonal relations, ability to empathize with playmates, and in resolution of hostile impulses. Although less severe disturbance was seen in children with a unipolar depressed mother, these children also differed from those from control families. Relative to controls, children with a unipolar depressed mother were unlikely to engage in physical aggression with their playmates, likely to show preoccupation and upset when exposed to conflict and distress in others, and likely to be somewhat less spontaneous in their expression of emotions. Comparisons will be made of the children's behavior at two and five years in order to explore processes associated with continuities and discontinuities in patterns of coping in children with an affectively ill parent.

Project Description:

The moderate degree of correspondence reported in offspring studies between the emotional problems of depressed parents and the problems of their children indicates the need to identify both those processes that contribute to patterns of intergenerational concordance in affective disorders and also those processes that contribute to nonconcordance. The offspring studies suggest also the need to examine the early evolution of symptoms in children, in order to identify possible precursors of diagnosable affective illness.

Key elements of adult depression include disturbances in regulation of emotion, problems in social relations, and feelings of helplessness, all of which may combine to produce lowered levels of social-emotional competence in a parent. Chronic exposure to such symptoms might be expected to influence children's own social-emotional functioning and to result, sometimes, in parallel problems to those of the parent. The first purpose of this research is to identify, early in development, adaptive and maladaptive patterns of social-emotional functioning in children with a depressed parent. The second purpose is to explore, through longitudinal assessments, the processes by which (a) early problems do or do not later culminate in diagnosable disturbances and (b) early social-emotional competence does or does not predict later adaptive functioning. To examine these issues, children's moods, social competence, psychiatric status, and capacities for social problem-solving, negotiation, and conflict resolution with peers are explored.

Methods Employed and Major Findings:

Young children of normal mothers and mothers with diagnoses of unipolar depression (current major depression, past major depression or past minor depression) are compared. DSM-III criteria were used to diagnose depression. The children were first observed as two-year-olds, in interaction with their mothers, adult strangers, and familiar playmates in laboratory settings. Their adaptive and maladaptive patterns of aggression, empathy, affiliative behavior and emotion regulation were examined under a range of experimental conditions designed to create pleasure, challenge, conflict, and distress. In an earlier study, a small sample of two-year-olds with a bipolar parent when exposed to these procedures showed multiple deficits in regulation of emotion. Less severe disturbance was seen in the children with a unipolar depressed mother, although these children, too, differed from children of control families. The children were especially sensitive to issues regarding physical and psychological injuries of others; they were less likely to engage in activities that might potentially bring physical injury to their playmates, and showed more preoccupation and upset when exposed to conflict and distress in others. This heightened emotionality sometimes momentarily disrupted their own social interactions. Children from control families, on the other hand, were more inclined to remain less involved with others' problems: Their efforts to help others, though no less frequent, more often took the form of guiding the person in distress away from the problem rather than attempting to deal with it directly.

Children with a unipolar depressed parent at times appeared already to be suppressing certain emotional tendencies. They were particularly polite in the face of frustration and seemed to make deliberate use of positive emotions to cope with some stressful situations. Not all of the children with an affectively ill parent showed problems and some children with normal parents showed problems.

Comparable research procedures are used to study these children again at age five. The Beck Depression Inventory and a mood scale are administered to both mothers and fathers. Mothers complete the Achenbach symptom checklist on the children and independent psychiatric assessments are made as well. Structured tests are used to measure, in hypothetical situations, dimensions of children's functioning that have parallels in the observational procedures (i.e., strategies for conflict resolution, social problem-solving abilities, moods, and empathy). Cognitive performance is also examined.

Significance to Biomedical Research and the Program of the Institute

Analysis of how children regulate their emotions, cope with stressful life events, negotiate interpersonal problems and develop close relationships will contribute to understanding of the etiology of affective problems in children, and will help to explain why mental illness persistently characterizes successive generations in some families and also how some children from disturbed families learn to master situations of conflict and distress.

Proposed Course:

The follow-up data collection on the psychiatric evaluation and the peer interactions of the five-year-olds is near completion. Coding systems and analytic strategies are being developed to examine patterns of continuity and discontinuity in children's social and emotional functioning.

Publications:

Zahn-Waxler, C., Cummings, E. M., and Cooperman, G. Emotional development in childhood. Annals of Child Development, Vol. I, JAI, 1984, 30-72.

Zahn-Waxler, C., Friedman, S., and Cummings, E.M.: Children's emotions and behaviors in response to infants' cries. Child Dev., 1983, 54, 1522-1528.

Zahn-Waxler, C., Radke-Yarrow, M., and King, R.: Early altruism and guilt. Acad. Psychol. Bull., 1983, 5, 247-259.

Zahn-Waxler C., Cummings, E. M., Iannotti, R. J., and Radke-Yarrow, M. Young offspring of depressed parents: A population at risk for affective problems. In D. Cicchetti and K. Schneider-Rosen (Eds.), New Directions for Child Development, Developmental Approaches to Childhood Depression. Jossey-Bass, Inc., San Francisco, in press.

Zahn-Waxler, C., Cummings, E. M., McKnew, D. H., and Radke-Yarrow, M. Altruism, aggression, and social interactions in young children of manic-depressive parents. Child Dev., 1984, 55, 112-122.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02156-05 LDP

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Personality Development of Children Reared by Normal and Depressed Mothers

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Marian Radke-Yarrow

Chief

LDP NIMH

COOPERATING UNITS (if any)

NONE

LAB/BRANCH

SECTION

Laboratory of Developmental Psychology

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland

TOTAL MAN-YEARS

.10

PROFESSIONAL

.10

OTHER

.00

CHECK APPROPRIATE BOX(ES)

 (a) Human subjects (b) Human tissues (c) Neither (a1) Minors (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The personality development of young children, on whom there is both genetic and rearing information, is investigated. The children are of normal parents and of depressed parents. Affective, social, and cognitive aspects of behaviors are examined. Children, initial ages 15- to 24-months and their siblings (5- to 8-years) are studied over a 3-year period. The several criteria guiding the selection of variables are: (a) critical developments in the transition from infancy to early childhood, and in the early school years, (b) interrelations of affective, social, and cognitive development, and (c) qualities presumed to be at risk in children of affectively ill parents. Personality is investigated relative to (a) the child's responsiveness to stimulation in the environment--new, pleasurable, and confrontational or aversive stimulation, (b) qualities of the child's affect and affect regulation, (c) qualities of the child's relationships, and (d) the child's sense of self. Work is progressing on conceptual and measurement issues.

Project Description:

The personality development of young children, on whom there is both genetic and rearing information, is investigated. The children are of normal parents and of depressed parents. Affective, social, and cognitive aspects of behaviors are examined. Children, initial ages 15- to 24-months and their siblings (5- to 8-years) are studied over a 3-year period. The several criteria guiding the selection of variables are: (a) critical developments in the transition from infancy to early childhood, and in the early school years, (b) interrelations of affective, social, and cognitive development, and (c) qualities presumed to be at risk in children of affectively ill parents. The research described in MH 021444 is the source of the data. Observational data are the primary data, supplemented by standard tests. The behavior sample is (a) extensive in time (9 hours), (b) varied in settings (newness, pleasure, play, frustration, stress, problem-solving, and (c) varied in social composition (child alone, with mother, sibling, stranger, unfamiliar peer, father, and family). Personality is investigated relative to (a) the child's responsiveness to stimulation in the environment: new, pleasurable, and confrontational or aversive stimulation, (b) qualities of the child's affect and affect regulation, (c) qualities of the child's relationships, and (d) the child's sense of self. An interest in this project is the influence of the child's personality in creating his/her own environment. Work is progressing on conceptual and measurement issues.

Significance to Biomedical Research:

The period of development from infancy to early childhood is a time when many behavioral and psychological qualities emerge and become organized within the individual. Early individual differences in these attributes have implications for the current mental health of the child and for the subsequent development of adaptive and maladaptive behaviors.

Proposed Course:

This part of the program project has received relatively little staff time, as related analyses within the project MH 02144 have gotten underway. Research development relating to child personality can now receive greater emphasis. Detailed development of the framework and the coding procedures will be underway during this year.

Publications:

Zahn-Waxler, C., Cummings, E.M., Iannotti, R.J., and Radke-Yarrow, M.: Young children of depressed parents. Zero to Three, National Center for Clinical Infant Programs, Spring Issue, 4: 7-12, 1984.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02158-05 LDP

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Impact of the Environment on the Development of the Abused Child

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Penelope K. Trickett	Senior Staff Fellow	LDP NIMH
Other:	Elizabeth J. Susman	Senior Staff Fellow	LDP NIMH
	Leon Kuczynski	Visiting Associate	LDP NIMH
	Malcolm Gordon	Research Psychologist	LDP NIMH

COOPERATING UNITS (if any)

NONE

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland

TOTAL ~~XXX YEARS~~ PERSON YEARS

1.70

PROFESSIONAL:

1.60

OTHER:

.10

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This study focuses on the emotional development of physically abused children and the relationship between this development and the childrearing environment of the home. While clinical evidence shows that abused children are at risk for a wide range of psychological problems, few controlled empirical studies exist and there is no research which relates aspects of the enduring environment of the abused child to the child's development. This study uses a multi-method approach to obtain information. The child's development is assessed in relation to cognitive and physical maturity, affective behavior, interpersonal problem solving skills, and behavior problems. The childrearing environment is assessed in terms of psychosocial environment of the home, family social network and social supports, parental frustration tolerance, childrearing attitudes and practices, and parental mood. Psychopathology in the parents is also assessed. Subjects are 4- to 10-year-old abused and control children and their families. Analyses focussing on children's misbehavior and parental discipline styles show that abused children commit more antisocial transgressions and are more oppositionally noncompliant than are control children. Also, abusive parents punish more frequently and use more severe forms of physical punishment while control parents use more simple request for compliance and reasoning. Early analysis of parental psychiatric status suggest a higher proportion of depression diagnoses for abusive parents than for non-abusive parents.

Project Description:

This study focuses on the psychological and behavioral development of physically abused children and the relationship between this development and aspects of the rearing environment of the child including the presence of psychopathology in the parents. The study is being conducted within a framework which includes characteristics of the abused child, the abusive parent, and the family environment and is based on the premise that both the causes of and the impact of child abuse can be understood only by considering physical abuse within the context of the enduring parent-child relationship or child-rearing environment.

One purpose of the study is to characterize the psychological development of abused children. While there is wide agreement that the childhood victims of physical abuse are at risk for later behavioral maladjustment, few controlled empirical studies bearing on this issue exist.

A second purpose is to investigate the childrearing environment of abusive families with particular attention to those processes which may lead to abusive incidents. There are competing hypotheses; (1) that abusive parents may believe that harsh punishment is a necessary technique if one is to rear a child adequately and (2) that abusive parents, while not valuing physical punishment any more than other parents, tend toward out-of-control anger episodes set off by child misbehavior. What contribution parental psychopathology may play in triggering abusive episodes is unknown and is a third focus of study.

A fourth purpose is to investigate relationships between the childrearing variables and the development of the abused child. As suggested earlier, the child's development is likely to be affected not just by the sporadic episodes of physical abuse, per se, but by the more enduring childrearing environment of the home.

Participants are physically abused children ranging in age from 4- to 10-years and their parents. They are recruited from protective service agencies in the Washington Metropolitan area. Criteria for inclusion in the sample are being either a single- or two-parent family in which at least one of the parents is the abuser. A control group of non-abusing families is recruited from community agencies. These families are matched to the abusing families on age, race, and sex of the child and educational and occupational status of the parents. The total sample is 56 families.

This study uses a multi-method approach to obtain information about the social and emotional development of the child and of the childrearing environment of the home. Standardized measures are used to assess the child's development in the areas of cognitive and physical functioning, social problem solving skills, and behavior problems. Affective coping style and predominant mode of relating to family members are assessed by observational methods.

To assess the childrearing environment, a combination of standardized measures and observational methods is used. The variables measured include family psychosocial environment, parental frustration tolerance, parental childrearing attitudes, values and practices, parent-child interaction, parental mood, and

parental psychiatric diagnosis (SADS). (See previous years' reports for details.) One set of completed analyses concern children's misbehaviors and parental discipline strategies of the abusive and non-abusive families. The data come from reports kept by the parents who were trained to observe and record naturally occurring incidents of discipline for five consecutive days in a standard format that preserved the sequence of parent and child behaviors.

Analyses of these reports indicate the following: (1) Compared with the control group children, the abused children were reported to commit more antisocial transgressions (unprovoked aggression, destructiveness, out-of-control anger) and, in reaction to parental discipline, were more noncompliant and more likely to accompany noncompliance with negativistic verbalizations. This may suggest both the contribution of the child to abusive incidents and the ineffectiveness of abusive parents' discipline attempts. (2) General differences were found in the frequency with which abusive and non-abusive parents used different disciplinary approaches: Abusive parents were more likely to use punitive strategies, both verbal and physical, and to use more severe forms of physical punishment than controls, while controls were more likely to use simple requests for compliance and reasoning. Moreover, control parents were more discriminating in their disciplinary approach, using different techniques depending on the nature of their child's misbehavior (which has been found to be an adaptive child-rearing technique in previous research) while abusive parents tended to use the same punitive techniques regardless of the situation. Disciplinary interventions were found to be equally stressful for both abusive and non-abusive parents in the sense of producing dysphoric feelings indicative of depression, anxiety, and doubt. However, abusive parents reported significantly more anger after these incidents than non-abusive parents.

Early analyses of parental psychiatric diagnosis (using the SADS) suggest a much higher proportion of depression diagnoses for abusive parents than for non-abusive parents.

Significance to Biomedical Research:

This study addresses two distinct etiological issues. One focus is on the causes of child abuse with particular emphasis on the role played by parental psychopathology and parental child-rearing attitudes and behavior. The second on the effect of child abuse on the psychological development focus is of the victims. Information on these two issues can aid greatly in the development of treatment programs for abusive families and preventive interventions.

Proposed Course:

Data collection is complete. The coding and analysis of the data continue. Two manuscripts, "Heterogeneity in Children's Responses to Similar Abusive Environments" and "Children's Misbehaviors and Parental Discipline Strategies in Abusive and Non-abusive Families" were completed and submitted for publication. Work on four other manuscripts is underway and the project should be completed within one to two years.

Publications:

Trickett, P.K.: The interaction of cognitive styles and classroom environment in determining first-graders' behavior. J. Appl. Develop. Psychol. 4: 43-64, 1983.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02159-04

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Information Processing and Adaptation to Research Hospitalization

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI : Elizabeth J. Susman Senior Staff Fellow LDP/NIMH

Other: Lorah D. Dorn Social Science Analyst LDP/NIMH
John C. Fletcher Special Assistant CC/DIR

COOPERATING UNITS (if any)

Office of the Director, Clinical Center

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland

TOTAL MAN-YEARS: Person Year PROFESSIONAL:

.50

.45

OTHER:

.05

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Children's participation in medical research has always presented special challenges. What is their level of understanding of the disease process and the treatment process? How is their understanding affected by their anxiety associated with hospitalization? How should their assent be interpreted in light of their various levels of understanding? This study focuses on a part of these interlocking issues (a) by comparing levels of understanding of 3 age groups (7-12, 13-18, 19-30), (b) by examining level of understanding of the disease and treatment processes in relation to standard measures of cognitive functioning - unrelated to disease, and (c) by assessing level of anxiety. These measures permit exploration of developmental differences in comprehension of one's medical situation, and allow examination of the effects of anxiety on comprehension at each of the age levels. A more general question, the effects of stress and anxiety on cognitive functioning, is addressed in the comparisons of the patient's level of cognitive functioning on the non-stressful content of the standard cognitive tests with his/her comprehension and reasoning on the stressful medical content. Participants are child, adolescent, and adult inpatients and a comparison group of healthy participants. Psychological assessments consist of standard cognitive tests, a test of anxiety level, and interviews. Preliminary findings show that age predicts participants' level of reasoning concerning stressful content related to their disease and treatment, but verbal abilities (Peabody, Picture Vocabulary Test) predict level of reasoning on a standardized test of knowledge of illness. Anxiety was associated with lower levels of reasoning on the non-stressful content.

Project Description:

Children's participation in medical research has always presented special challenges. What is their level of understanding of the disease process and the treatment process? How is their understanding affected by their anxiety associated with hospitalization? How should their assent be interpreted in light of their various levels of understanding? This study focuses on a part of these interlocking issues (a) by comparing levels of understanding of 3 age groups (7-12, 13-18, 19-30), (b) by examining level of understanding of the disease and treatment processes in relation to standard measures of cognitive functioning - unrelated to disease, and (c) by assessing level of anxiety. These measures permit exploration of developmental differences in comprehension of one's medical situation, and allow examination of the effects of anxiety on comprehension at each of the age levels. A more general question, the effects of stress and anxiety on cognitive functioning, is addressed in the comparisons of the patient's level of cognitive functioning on the non-stressful content of the standard cognitive tests with his/her comprehension and reasoning on the stressful medical content. Participants are child, adolescent, and adult inpatients and a comparison group of healthy participants. Psychological assessments consist of standard cognitive tests, a test of anxiety level, and interviews.

Participants in the study are children (7-12 years old), adolescents (13-18 year olds), and young adults (19-30 year olds) who are admitted to the Clinical Center for the first time. The patients are enrolled in medical protocols involving either the treatment of obesity or the treatment of childhood cancer. A comparison group of healthy non-hospitalized participants matched for age, sex, and socioeconomic status also is included. Seventy-five participants will be included in the study. Level of reasoning and understanding about their illness and treatment regimens is obtained through interviews. Standard tests of cognitive abilities and reasoning about non-stressful content also are administered. Anxiety is measured using the Spielberger State-Trait Anxiety Scale. Participants are retested after 6 months. Preliminary findings show that different factors predict level of reasoning concerning stressful content related to one's own disease, and level of reasoning about content on a standardized cognitive test of knowledge of illness. Chronological age of participant was the best predictor of level of reasoning concerning stressful content, but verbal ability (Peabody, Picture Vocabulary Test) was the best predictor of level of reasoning on the test of knowledge of illness. Anxiety was associated with lower levels of reasoning on the test of knowledge of illness.

Significance to Biomedical Research:

Level of comprehension of the disease and treatment and level of anxiety are significant factors in the behavior problems and noncompliance frequently observed in hospitalized children and adolescents. Systematic data on these factors can aid in developing effective methods of communicating complex medical information to children and of dealing with their anxieties.

Proposed Course

The data are being analyzed.

Publications:

Blumberg, B.D., Lewis, J., and Susman, E.J.: A time of transition. In Eisenberg, M.G. and Jansen, M.A. (Eds.): Impact of Chronic Disabling Conditions on Self and Family. New York, Spring Press, 1983, pp. 133-150.

Susman, E.J. and Hollenbeck, A.R.: Sequential variations in the interactions between caregivers and child and adolescent cancer patients. International Journal of Behavioral Development. In press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02161-04 LDP

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Developmental Changes in Imitative Learning

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Leon Kuczynski	Visiting Associate	LDP/NIMH
Other:	Carolyn Zahn-Waxler	Research Psychologist	LDP/NIMH
	Marian Radke-Yarrow	Chief	LDP/NIMH

COOPERATING UNITS (if any)

NONE

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland

TOTAL MAN-YEARS: Person Year

.35

PROFESSIONAL:

.30

OTHER:

.05

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This study is concerned with the development of children's imitative behavior in the natural environment. Data were obtained on 24 children over a nine-month period during the second and third years of life. Sources of data consisted of descriptive accounts of imitation by mothers trained in observational recording. Although most of the incidents of imitation consisted of immediate repetitions of the behavior of models, incidents of delayed imitation increased from late infancy to early toddlerhood. Several developmental changes in the content of children's imitations also occurred during this period. The imitation of positive affect and of nonfunctional behaviors decreased as children grew older. Increases with age were found for caretaking, self-care, and household chore behaviors, social interaction skills, mannerisms and expressive characteristics of models, and verbal behaviors. The overall pattern of findings suggests that imitation is an important process in the early acquisition of competent behavior patterns and that the imitation of instrumental and functional behavior patterns increases with age.

Project Description:

The early development of children's imitative behavior in the natural environment is investigated. Children's imitation of parents, siblings, and other models is considered an important process in children's acquisition of complex patterns of behavior. Previous laboratory research with pre-school and older children suggests that aggressive, prosocial, self-controlled, and impulsive patterns of behavior may be promoted by imitation. However, the primary focus of research has been on the experimental manipulation of variables such as the characteristics of the model or of the situation that govern the process of imitation. Information is lacking about the kinds of behaviors that children spontaneously imitate in the natural environment and about the role of imitation in the learning of children in the first years of life.

The present study extends previous research by investigating the development of both immediate and delayed forms of imitation as it occurs in natural settings. One source of data was detailed narrative accounts of children's behaviors recorded by mothers trained in observational procedures. Reliabilities of maternal reports were assessed by comparing mothers' and investigators' reports of children's imitations during home visits (percent of agreement was 91%). Mothers were also interviewed every three weeks during the data collection period and were asked to report new forms of imitation that had occurred since the preceding contact. Data were obtained on 24 children during a nine-month period. For half the children data were collected before the age of 17 months, for the other half, data were collected after the age of 17 months.

The analyses indicated that during the second year of life children's imitative repertoires are extensive and grow increasingly complex. Several developmental changes in the behaviors imitated by children indicate an increasing tendency for children to imitate behaviors that represent functional and meaningful achievements from the standpoint of socialization. Two categories of imitation decreased with age. Positive emotional expressions and discrete, nonfunctional behavior of models were more frequently imitated before the age of 17 months than later. Significant increases with age occurred for imitations of instrumental behaviors such as caretaking, self-care and household chores; interpersonal communication skills; mannerisms and expressive characteristics of the model and verbal expressions.

More detailed analyses of two categories of imitation--parental discipline and emotions--also indicate a possible development from simple, discrete repetitions to more organized, functional patterns of behavior. For example, although there were no overall age differences in children's imitation of parental behaviors during disciplinary interventions, there was a change in the function of these imitations. Whereas younger children were more likely simply to repeat parental reprimands without directing them to others, older children were more likely to direct their imitations to others. Similarly, although imitations of emotions, in general, decreased with age, there was an increase in the extent to which these imitations had a contrived quality.

Although most of the imitations in this study consisted of immediate repetitions of the behavior of models, there was a significant increase in the incidence of delayed imitations with age. This finding suggests that exposure to models may have more lasting effects on children's memory and learning of new patterns of behavior as children grow older. In summary, this study suggests that imitation is an important process in children's personality development and particularly in the acquisition of instrumental and social skills. Moreover, the patterns of developmental changes indicate that children's imitation of functional patterns of behavior increases from late infancy to early toddlerhood.

Significance to Biomedical Research:

Imitation is a basic process of learning and has obvious implications for the environmental transmission of complex patterns of behavior. An inherent aspect of the environment of children living with parents suffering from psychopathology is the presence of models of disordered patterns of behavior and affective expression. Although few studies have investigated what behaviors are susceptible to imitation, disordered forms of parental behavior may, in part, be transmitted by this process. This study makes a start in assessing the impact of parent and sibling models by examining the content of imitated behaviors in early childhood.

Proposed Course:

A report of these findings has been submitted for publication.

Publications:

None

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02164-04 LDP

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Impact of Biological Changes on Psychological Functioning During Adolescence

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI	: Elizabeth J. Susman	Senior Staff Fellow	LDP/NIMH
Other:	Editha Nottelmann	Senior Staff Fellow	LDP/NIMH
	Gale E. Inoff	Research Psychologist	LDP/NIMH
	Lorah Dorn	Social Science Analyst	LDP/NIMH
	George P. Chrousos	Senior Investigator	DEB/NICHD
	Gordon B. Cutler	Senior Investigator	DEB/NICHD
	D. Lynn Loriaux	Chief	DEB/NICHD

COOPERATING UNITS (if any)

Developmental Endocrinology Branch

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland

TOTAL ~~XXXXXX~~ Person Years PROFESSIONAL

2.60

2.45

OTHER:

.15

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This research examines the interrelations of endocrine and physical growth variables and adolescents' psychological functioning. Participants are girls and boys, 9- to 14-year-olds, and their parents. Participants are evaluated on biological and psychological variables at three times of measurement, six months apart. Biological measures include stage of pubertal development, assessed by a physical examination (Tanner stages) and hormone levels, assessed by blood samples for gonadotropins, gonadal steroids, and adrenal androgens. Psychological measures include assessments of psychological and behavioral problems, cognitive functioning, self-esteem, affective states and interpersonal functioning. Assessment of parental behavior is through observations of parent-child interactions in standard laboratory situations and through standard inventories regarding childrearing attitudes and behavior. Data from the first time of testing indicate clear and consistent relationships between endocrine status and psychological functioning. For girls and boys, hormone levels indicative of a more mature endocrine status were related to feelings of low competence and negative self-image. Mothers of girls with lower levels of adrenal androgens report more anxiety, depression, withdrawal, aggression, and cruelty than mothers of girls with higher levels of adrenal androgens. Mothers of boys with the higher levels of sex steroids and adrenal androgens report more obsessive compulsive, delinquent, aggressive, hyperactive, schizoid, and uncommunicative behaviors and somatic complaints. Future analyses of the longitudinal data will examine the causal relations between changes in endocrine status and changes in psychological functioning of adolescents.

Project Description:

This research examines the interrelations of endocrine and physical growth changes and adolescents' psychological functioning. Cognitive, affective, and interpersonal competencies and dysfunctions are assessed. The objectives of this research are to examine: (a) the interrelations among these psychological processes in early adolescence; (b) the relations between these psychological processes and biological variables (endocrine and physical growth levels and changes); and (c) the interrelations of childrearing variables, biological development, and psychological functioning in early adolescence. Each objective is approached cross-sectionally as well as longitudinally by assessing each child three times at six-month intervals over a period of one year.

Methods and Findings:

Equal numbers of boys (ages 10 to 14) and girls (age 9 to 13) were selected at each of the five stages of pubertal development as defined by Tanner. Approximately 100 adolescents and their parents, working class to professional class, are in the study. Biological and psychological assessments are made at three times of measurement. Blood levels of gonadotropins, sex steroids, and adrenal androgens as well as height, weight, head circumference, and stage of pubertal development are obtained. Psychological measures include: cognitive tests, self-ratings of daily moods and perceived instigations of mood changes, self-image and self-esteem, adolescents' reports on relationships with peers and family, and academic achievement. Parallel data are obtained from the parents. Parents also report on their childrearing attitudes and practices. Two measures aimed specifically at identifying problem behaviors are a psychiatric interview (Diagnostic Interview for Screening Children) and the parent's assessment of the adolescent's behavior problems (Achenbach Child Behavior Checklist). The adolescents and their parents come to the Laboratory where most of the measures are completed and where parent-adolescent interaction is observed. The parents and adolescent work together on conflict-resolution tasks (e.g., how to handle family problems and disagreements regarding personal characteristics of the adolescent). The interactions are videotaped.

Data from the first time of testing indicate clear and consistent relationships between endocrine status and psychological functioning. Girls with higher levels of gonadotropins and sex steroids feel less competent, cognitively, socially, and physically, than girls with lower levels. Girls with higher levels of gonadotropins, sex steroids and adrenal androgens have a poorer image of themselves with respect to social relationships, impulse control, and depression. Similarly, boys with higher levels of gonadotropins and adrenal androgens feel less competent than boys with lower levels. Boys with higher levels of adrenal androgens reported problems in social relations, impulse control and feelings of mastery over the external world. In summary, for girls and boys, hormone levels indicative of a more mature endocrine status were related to feelings of low competence and negative self-image. Girls' self-presentations differ from assessments made by their mothers. Mothers of girls with lower levels of adrenal androgens report more anxiety, depression, withdrawal, aggression, and cruelty than mothers of girls with

higher levels of adrenal androgens. Mothers of boys with the higher levels of sex steroids and adrenal androgens report more obsessive compulsive, delinquent, aggressive, hyperactive, schizoid, and uncommunicative behaviors and somatic complaints.

Future analyses of the longitudinal data will examine the causal relations between changes in endocrine status and changes in psychological functioning of adolescents.

Significance to Biomedical Research:

The increase in behavior problems during adolescence has been documented extensively but there are many questions regarding the etiologies of these problems. Findings from this study can help to clarify the interrelations among endocrine factors, parental childrearing practices and adolescent psychological characteristics. Findings will have implications for prevention and intervention programs.

Proposed Course:

Data collection is complete for times one and two, and 80% for time three. The data are being analyzed and manuscripts are being prepared.

Publications:

Inoff, G.E., Halverson, C.F., Jr., and Pizzigatti, K.A.: The influence of sex-role stereotypes on children's self- and peer-attributions. Sex Roles 9: 1205-1222, 1983.

Hamburg, B.A. and Inoff, G.E.: Coping behaviors in diabetes: Relationships between knowledge of diabetes, locus of control, and metabolic control. In Ahmed, P. (Ed.): Living with Juvenile Diabetes. Springfield, Illinois, Charles C. Thomas, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02165-02 LDP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Adjustment in Early Adolescence: Family and Peer Influences

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Editha D. Nottelmann

Senior Staff Fellow

LDP/NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland

TOTAL MANPOWER: Person Years PROFESSIONAL:

.25

.20

OTHER:

.05

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The study focuses on family and peer influences in the transitional period of early adolescence, in which children experience multiple change and therefore are subject to stress. Supportive relationships are thought to act as moderators of stress for adults, but no data exist on the contribution of supportive relationships to psychological adjustment in early adolescence. Assessments focus on family and peer relations and peer-adult networks in early adolescence.

Project Description:

The study examines family and peer influences on psychological adjustment in early adolescence. Early adolescence is a period of considerable stress and vulnerability. It also is a period when children are taking the first steps away from dependence on their parents and beginning to seek psychological support from their peers. Supportive relationships are thought to protect against vulnerability to stress. Failure to establish and maintain supportive relationships are likely to heighten the risk for poor adjustment and serious problem behavior. Assessments focus on the the contributions of family and peer relationships and peer-adult networks to children's psychological adjustment and adaptation to change.

The sample of 162 early adolescents and the interview instrument was described in last year's report. Briefly, it consisted of 81 boys and 81 girls, half of whom had completed Grade 6 and the other half Grade 7 at the time of the interview. The average 6th-grader was in early puberty, the average 7th-grader was in mid-puberty; and the girls in each grade were farther advanced into puberty than the boys.

No significant grade or sex differences were found in the personal social networks (persons not living in the same household) of these early adolescents for (a) structural properties: total number of persons ($\bar{M} = 6.70$), total number of adults ($\bar{M} = 2.00$), total number of children ($\bar{M} = 5.46$); (b) kinship status of social network members: number of adults who are members of the immediate family ($\bar{M} = .14$), number of adults who are members of the extended family ($\bar{M} = 1.47$), number of unrelated adults ($\bar{M} = .51$), peers who are members of the extended family ($\bar{M} = .46$), and unrelated peers ($\bar{M} = 4.93$); or (c) network density, the number of network members who know each other ($\bar{M} = 5.13$). Similarly, there were no significant differences in the number of persons living in the same household ($\bar{M} = 4.30$) or in the number of siblings living at home ($\bar{M} = 1.74$).

However, there were significant grade and sex differences in the responses of these early adolescents to questions about the quality or closeness of their relationships, in which immediate family members living in the same household were included. These were questions about who understands them best, whom they would go to for help, whom they would like to talk things over with if they had to choose one person, and whom they would want to be like. Generally, the responses revealed a greater parent than peer orientation in the younger than older group, primarily among boys, and a greater peer than parent orientation among the older group, primarily among girls. The age differences were striking, even though the younger and older groups are only one year apart, as were the sex differences. They suggest a shift from parents to peers, with girls in the lead, that is related to physical maturation. Also noteworthy is the finding that when a parent was nominated, it usually was the mother. Fathers were nominated most often together with mothers--usually by boys, rarely by themselves. Girls rarely mentioned their father. Fathers were nominated most frequently by boys (20%) when they were asked whom they would want to be like.

For activities with their parents, there were significant sex differences only in the number of things adolescents reported doing with their father.

Boys reported more activities ($\bar{M} = 2.67$) than girls ($\bar{M} = 1.28$). There were no significant grade or sex differences in the number of things adolescents reported doing with their mother (about 3) or with both parents (about 5).

For activities with peers, there were significant grade and sex differences in the number of activities away from home, with the older adolescents, particularly girls, doing more things away from home and more often getting together with their friends than the younger adolescents.

Significance for Biomedical Research:

The factors contributing to, or protecting against disordered affect and problem behavior in adolescence are poorly understood. This study will generate information about the contributions of family and peer influences to children's psychological functioning.

Proposed Course:

A first manuscript is in preparation:

Nottelmann, E. D., & Welsh, C. J. Social networks in early adolescence.

Data evaluation is continuing. Further manuscripts are planned, including articles on adolescent concerns, and a comparison of parent and adolescent perspectives on adolescent social relationships.

Publications:

None

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02166-02 LDP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Developmental Patterns of Cognition and Interaction in Children at 2 and 5 Years

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI : Blaise Pierrehumbert Guest Worker LDP/NIMH

Other: Ronald J. Iannotti Research Psychologist LDP/NIMH
 David Pellegrini Assistant Professor Catholic University

COOPERATING UNITS (if any)

Catholic University, Washington, D.C.

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland

TOTAL MAN-YEARS

.30

PROFESSIONAL:

.26

OTHER:

.04

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

One possible contribution to maladaptive or aberrant behaviors of children is a cognitive component; namely, distorted perceptions of their social, personal, and physical environments and distorted causal attributions regarding their responsibility for these events. The aims of this project are to develop measures of the cognitive capabilities (social perspective taking, and causal attributions of physical, social and affective events) and to examine relations between these cognitive variables and interpersonal behavior. Two samples of children were studied. Forty-eight were seen at 2 years and 5 years. A second sample (N = 35, 4 to 11-year-olds) was seen only once. Mothers in both samples had been given a psychiatric screening interview (SADS); the mothers in the study had diagnoses of either "normal" or "depressed". The children were observed in standard play sessions with a familiar peer; mothers were present in the sessions. Interactions were evaluated for frequency of initiations, social responsiveness, and complexity of each type of interaction. Perspective-taking was assessed at 2 years. Causal attribution measures were given at the later ages. The mother-child relationships at two years were positively related to child-mother and child-peer relationships at five years. Perspective-taking ability at 2 years was positively related to measures of empathy and internal locus of control at 5 years. Mother's diagnosis was unrelated to cognitive measures in the children. Children with problematic behavior differed from the children without problems on their causal attributions; they were more likely to perceive themselves at fault for negative events in their lives.

Project Description:

One possible contribution to maladaptive or aberrant behavior of children is distorted preceptions of their social, personal, and physical environments and distorted causal attributions regarding their responsibility for these events. Children's perceptions of physical and social events, their causal attributions regarding responsibility for these events, and the relations between these abilities and children's interpersonal behaviors were investigated.

Two samples of children were studied. Forty-eight were seen at 2 years and 5 years. A second sample (N = 35, 4 to 11-year-olds) was seen only once. Mothers in both samples had been given a psychiatric screening interview (SADS); the mothers in the study had diagnoses of either "normal" or "depressed". The children were observed in standard play sessions with a familiar peer; mothers were present in the sessions. Interactions were evaluated for frequency of initiations, social responsiveness, and complexity of each type of interaction. Perspective-taking was assessed at 2 years. Causal attribution measures were given at the later ages.

The mother-child relationship at two years was positively related to child-mother and child-peer relationships at five years. Perspective-taking ability at 2 years was positively related to measures of empathy and internal locus of control at 5 years. Children's causal attributions of physical, social, and affectional events were intercorrelated. These cognitions are unrelated to the child's relationships with mothers and peers and unrelated to mothers' diagnoses. However children with problems differed from non-problem children in their attributional processes; they were more likely to perceive themselves at fault for negative events in their lives.

Significance to Biomedical Research:

The cognitive components of children's adaptive and maladaptive interpersonal behaviors have tended to be ignored, while emphasis has been on overt behavior patterns. This research contributes tools for assessment of cognitive processes and provides information concerning cognition-behavior relationships.

Proposed Course:

Results have been presented at scientific meetings and manuscripts have been submitted for publication:

Pierrehumbert, B., Iannotti, R.J., and Cummings, E.M.: Maternal attachment and social development in children between two and five years. Presentation at:

Faculte de Psychologie, Universite de Geneve, May 1984.

Service Universitaire, Vaudois de Psychiatrie Infantile, Lausanne, Switzerland, May 1984.

Centre Hospitalier, Universitaire Vaudois, Lausanne, Switzerland, October 1984.

This is a final report.

Publications: None

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02167-02 LDP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Interpersonal Inferential Abilities in Normal and Depressed Mother-Child Pairs

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Ronald J. Iannotti	Research Psychologist	LDP NIMH
OTHER:	Carolyn-Zahn Waxler	Research Psychologist	LDP NIMH
	E. Mark Cummings	Senior Staff Fellow	LDP NIMH

COOPERATING UNITS (if any)

NONE

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland

TOTAL MAN-YEARS

Persons Years

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.30	.25	.05

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The self-preoccupation and egocentrism sometimes observed in affectively ill adults may significantly interfere with their ability to understand the experiences and needs of other persons. Such a deficit in a parent could seriously interfere with rearing functions. The purpose of this research is (a) to examine role-taking and interpersonal problem-solving abilities of depressed and non-depressed mothers, (b) to examine the development of parallel abilities in their children in the first years of life, and (c) to explore correspondences between parents' and children's capabilities and handicaps in making appropriate inferences about others' psychological states. Mother's abilities to make inferences about others' internal states are being assessed in hypothetical social problem-solving situations and in structured interactions with their child. Children's abilities are assessed in both natural and experimental environments.

Project Description:

The self-preoccupation and egocentrism sometimes observed in affectively ill adults may significantly interfere with their ability to understand the experiences and needs of other persons. Such limitations in a parent could seriously interfere with effective childrearing. If the parent has little awareness of others, including the child, the consequence is likely to be little ability to understand or empathize with the child. Sometimes paradoxically, depression may have the opposite effect of heightened sensitivity to others' needs. The purpose of this research is (a) to compare depressed and non-depressed mothers in their capabilities in making appropriate inferences about others' intentions, motives, and feelings, (b) to examine the development of parallel abilities in their young children, and (c) to explore correspondences between parents' and children's capabilities and handicaps in making appropriate inferences about others' psychological states. Comparisons of children of depressed mothers who are sensitive in interpersonal problem-solving abilities with children of mothers who are not may aid in understanding why some children with an affectively ill parent show adequate psychosocial adjustment while others develop behavior problems.

Methods:

Forty-eight mother-child pairs were studied. Children were 2 to 2-1/2 years of age. Mothers had DSM-III diagnoses of major depression, minor depression, or normal. Parental abilities to make inferences about internal states of others were assessed in standard hypothetical social problem-solving dilemmas and in structured interactions with their child. The children's cognitive (spatial, social, and affective) awareness was assessed in both natural and experimental environments. Experimental contexts were designed to elicit a range of interpersonal functioning (e.g., responding to a familiar peer who is distressed by the absence of his/her mother; responding to two adult strangers in a verbal argument, etc.). Children's reactions are evaluated for evidence of a child's social sensitivities.

Significance to Biomedical Research:

The present research will provide information concerning rearing experiences which either predispose the child to difficulties in interpersonal functioning or provide the child with training in effective interpersonal skills.

Proposed Course:

Analyses are proceeding on this data and will be completed during the coming year.

Publications:

Iannotti, R.J.: Naturalistic and structured assessments of prosocial behavior in preschool children: The influence of empathy and perspective taking. Dev. Psychol., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02169-02 LDP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Interactions Between Siblings With a Depressed Parent

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI	Carolyn Zahn-Waxler	Research Psychologist	LDP NIMH
Other:	Dale Hay	Guest Worker	LDP NIMH
	Marian Radke-Yarrow	Chief	LDP NIMH

COOPERATING UNITS (if any)

NONE

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland

TOTAL MAN-YEARS: Person Years PROFESSIONAL:

.22

.20

OTHER:

.02

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this research is to examine contributors to systematic differences in siblings in the degree to which their relationships with each other adaptively or maladaptively focused. Some siblings fight frequently while others develop good social relations. Siblings with normal and depressed mothers are compared. If a mother is depressed and less able to care for her children, some of the responsibility for caregiving and mediation of conflicts may fall to the older sibling. This would have implications for the psychosocial development of both the older and the younger sibling. Siblings are observed in interaction alone with each other, individually with the mother and together with the mother. The younger child is between the ages of two and three and the older child is between the ages of five and seven. Coding systems are currently being developed in which episodes of caregiving and conflict are identified and analyzed in relation to maternal psychopathology.

Project Description:

Neops psychoanalytic conceptualizations of interpersonal relations and affective communication patterns between siblings have focused principally on issues of jealousy and rivalry inherent in sibling interactions. Anthropological research has emphasized the adaptive significance of siblings in the socialization process: that is, relationships between siblings are characterized by nurturance and caretaking as well as by jealousy and aggression. Ethologists (e.g., Hinde) define the sibling system as one in which the potential for both altruism and aggression is heightened. The purpose of this research is to examine contributors to systematic differences in siblings in the degree to which their relationships with each other are adaptively or maladaptively focused. Some brothers and sisters fight frequently and chronically while others get along and take care of each other. It would be important to learn some of the reasons why this is so. Siblings with normal and depressed mothers are compared. If a mother is depressed and less able to care for her children, some of the responsibility for caregiving and mediation of conflicts may fall to the older sibling. This would have significant implications for the psycho-social development of both the older and the younger sibling. For example, for the older child it may foster resentment and/or it may heighten the development of precocious caregiving skills that ultimately may be either adaptive or maladaptive. For the younger child it may represent exposure to incompetent caregiving or needed protection from a dysfunctional parent. Reports in the literature suggest relatively high levels of conflict between siblings with a depressed mother (Weissman).

Methods and Results:

In a laboratory setting designed to approximate closely the natural rearing environment (see Z01 MH 02144), siblings are observed in interaction alone with each other, individually with the mother, and together with the mother. The younger child is between the ages of two and three and the older child is between the ages of five and eight. In this study, unlike many other studies of parent-child interaction, the family members are not only observed while playing together, but while trying to accomplish other things: the mother must serve a snack, the older sibling must watch over the younger while the mother is out of the room, the children must each attempt to solve challenging and potentially frustrating problems, and the family must reunite after the children have had separate stimulating experiences.

To tap the harmonious and disharmonious dimensions of family relations in these differing contexts, two general types of interactions are being examined: (1) caregiving episodes in which one family member (mother, older sibling, or younger sibling) meets an explicit or implicit need of another, and (2) conflict episodes in which one family member protests, resists, or retaliates against another person's action, i.e., situations in which their needs clash. These two types of episodes will reveal the social skills possessed by all three family members (e.g., roletaking, abilities to negotiate solutions to

interpersonal problems, etc.), and they will offer opportunities for the display of prosocial and aggressive actions. Furthermore, the patterning of caregiving and conflict episodes, as well as the content of the particular actions taken by the siblings and the mother, may be used to characterize each person's interactive style, and may be analyzed in relation to maternal psychopathology.

Significance to Biomedical Research and the Program of the Institute:

The ability to care for others with competence and empathy, both within and outside the family, is considered to be a marker of mental health (Spitzer and Endicott). It represents the reverse side of mental illness, which characteristically requires being cared for by others. Factors contributing to positive and negative caregiver capacities may be better understood by studying the phenomenon from a developmental perspective in families with and without emotionally disturbed caregivers.

Proposed Course

A coding system has been developed to characterize conflict and caregiving episodes between siblings. Codes have been developed that can be used across the differing interactive contexts but that are also sensitive to the unique demands of each challenge that the family members face. Episodes of conflict and caregiving between siblings of depressed and non-depressed mothers will be compared and examined in relation to parallel characteristics of mother-child interaction in the coming year.

Publications:

Zahn-Waxler, C., McKnew, D.H., Cummings, E.M., Davenport, Y.B., and Radke-Yarrow, M.: Problem behaviors and peer interactions of young children. Am. J. Psychiatry 141: 236-240, 1984.

Zahn-Waxler, C., Hollenbeck, B. E., and Radke-Yarrow, M.: The origins of empathy and altruism. Review of Animal Welfare Science and Philosophy, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02170-02 LDP

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Psychiatric Evaluation of Infants and Toddlers

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Leon Cytryn	Research Psychiatrist	LDP/NIMH
Other:	Donald McKnew	Research Psychiatrist	LDP/NIMH
	Tracy Sherman	Guest Researcher	LDP/NIMH
	Marian Radke-Yarrow	Chief	LDP/NIMH

COOPERATING UNITS (If any)

NONE

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland

TOTAL MAN-YEARS: PERSON-YEARS PROFESSIONAL:

1.50

1.45

OTHER:

.05

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Reliable psychiatric assessment procedures for use with young children are needed in clinical practice and in research. Procedures for children of these ages have been developed as part of a study of normal and depressed mothers and their offspring. Two kinds of instruments have been developed. The first relies on the child-clinician dyad in a semi-structured play interview. Assessment is in terms of areas of concern (following a standardized set of categories) and of DSM III diagnoses. A second instrument brings together the strengths of clinical psychiatry and child development. Mother and child are observed together in standard situations that involve conditions and requirements that are natural parts of the child's life (stress, closeness, pleasure, mother-unavailability, frustration, etc.). The situations are sequenced to comprise a reasonable script of events. Behaviors are coded by the clinician; the analysis combines a prescribed set of coding judgments, and clinical assessments. The sample consists of 102 children, 1 1/2 to 5 years. The assessments from the two procedures are being compared and evaluated against a number of criteria within the larger study (Project Z01 MH 02156) of child development in families with and without psychopathology.

Project Description:

Affective illness in latency age children and adolescents has been given much attention. However, most studies have bypassed the toddlers, and reliable diagnostic instruments for use with young children have not been available. Although it may not be possible to make exact diagnoses at this age, one would expect to find disturbances which might conceivably be precursors of future psychopathology. Findings of such precursors would contribute to a developmental view of affective illness and could be crucial to any attempts of secondary prevention.

Two kinds of psychiatric assessment procedures for children 1 1/2 to 5 years are being developed and evaluated. The toddlers who are being evaluated are part of a childrearing project in which the children of 4 groups of mothers are being studied (major depression, bipolar disorder, minor depression, and normal) (see Project Z01 MH 02144). There are 102 families.

The first procedure is a semi-structured play interview consisting of three 10-minute segments: free play with neutral toys (blocks, crayon and paper, ball, doll, teddy bear), play with family toys (doll house and small human figures whose age and sex duplicate the true family constellation), and aggressive play with guns, soldiers, boxing gloves, punching bag, and a pounding block. Before the interview begins, separation from the mother is observed and noted. In each play segment, the child is encouraged to use the toys in any manner he or she chooses. Running notes are kept by the examiner and each session is recorded on video cassette. Following the session, the child's performance is rated on a mental status scale and on 26 areas of concern (e.g. excessive shyness, difficulty in handling aggression, speech problems).

The second assessment procedure brings together some of the strengths of the clinician and the child developmentalist. The views and assessments of child behavior generally reflect the concepts and tools of the specific disciplines. The clinician's usual sources of information about the child tend to be the clinical interview and/or the child's presenting problems. Rarely is systematically observed behavior the data source. The developmental psychologist, on the other hand, has relied on structured interviewing of the mother and on direct and systematic observation of the child.

In this procedure, mother and child are observed in six standard situations that involve conditions and requirements that are natural parts of the child's life, and that tap the child's coping skills, mood regulation, and relationship with mother. Rather than the typical "free play" situation, variations in situations involve stress, relaxation, pleasure, commonality of activity, mother unavailability, and ambiguity. The situations are a part of the Rearing Study (MH 02144). They are also being tried in the clinician's office as a 45 to 60 minute procedure.

The skills of clinician and developmental psychologist are combined in the analytic scheme: The situations are viewed as episodes of interaction (in contrast to minute sequential analyses). The coders are child psychiatrists.

Each episode is scored first for the presence or absence of specific critical behaviors. Then clinical judgments of the child's functioning as either appropriate or not appropriate are made on eight scales: level of motor activity, content of thought, social attentiveness, social responsiveness, social initiation, coping and mastery, and emotional expression and regulation. The behavior is not scored against a presumed age norm (since age norms do not exist for these behaviors). Instead, the rater views the child as an ageless child. From these scores, a summed score for each scale across episodes is obtained.

A clinical assessment is then made of areas of concern in the child's behavior, the child's defenses or coping strategies, the child's risk status, and diagnosis. Treatment recommendations are made.

Significance to Biomedical Research:

The significance of this research is multifold: (1) The development of improved assessment instruments will help us to understand the developmental patterns of adaptation and maladaptation in very young children with limited language ability, and will permit more sensitive evaluation of the child's strengths and vulnerabilities. (2) These assessments will enable us to see whether patterns of adaptation differentiate between the children reared by normal and depressed mothers. (3) We will be able to evaluate how assessments from this perspective compare with assessments made in a standard child psychiatry playroom interview. Patterns of adaptation and maladaptation observed in the child, while not satisfying conditions of a DSM-III diagnosis, may be indices of vulnerability to the later occurrence of problems. This prospective information not only adds to our understanding the developmental course of affective illness, but may provide an informed basis for identifying children who are most at risk. The children are now being seen in a two-year follow-up.

Proposed Course:

The first instrument has been administered to the entire sample and coding has been completed. The second coding instrument has been constructed and half of the sample has been coded. Inter-rater reliability for the presence or absence of specific behaviors is 85%; for the clinical judgment scales, approximately 90%. The assessments from the two procedures are being compared and evaluated against a number of criteria within the larger study (see Projects Z01 MH 02144 & 02156) of child rearing and child development. Several kinds of scientific papers will be prepared: those that address issues of assessment methodology, and those that compare psychiatric status of children in families with and without parental psychopathology.

Publications:

Cytryn, L., Gershon, E. S., McKnew, D. H.: Is childhood depression a genetic illness. Integrative Psychiatry, 2: 17-23, 1984.

Cytryn, L., McKnew, D. H., Zahn-Waxler, C., Radke-Yarrow, M., Gaensbauer, T. H., Harmon, R. J., Lamour, M.: A developmental view of affective disturbances in the children of affectively ill parents. Am. J. Psychiatry, 141: 219-223, 1984.

Gaensbauer, T. J., Harmon, R. J., Cytryn, L., McKnew, D. H.: Social and affective development in infants with a manic-depressive parent. Am. J. Psychiatry, 141: 223-230, 1984.

Cytryn, L., McKnew, D. H., Zahn-Waxler, C., Gershon, E. S.: Developmental issues in risk research: The offspring of affectively ill parents. In Rutter, M., Izard, C. E., Read, P. B. (Eds.): Depression in Children: Developmental Perspectives. New York, Academic Press, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02171-01 LDP

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Protective and Risk Factors in Childrearing: Contributions of Fathers

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	W. Elbert Wilson	Research Psychologist	DRG/NIH
Other:	Marian Radke-Yarrow	Chief	LDP/NIMH

COOPERATING UNITS (if any)

Division of Research Grants
National Institute of Health

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland

TOTAL MAN-YEARS	PERSON-YEARS	PROFESSIONAL:	OTHER:
.65		.55	.10

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The research focuses on paternal contributions to child development. In particular, the role of fathers with wives with affective disorders is examined. Families are selected for the study on the basis of the mothers' diagnoses, as normal or depressed. Fathers, mothers, and two children (ages 2-5 & 8-11) are studied in a home-like environment established in the laboratory. They participate in a variety of planned situations representative of day-to-day family events. These combine and recombine the family unit into dyads and tryads as well as total group. Data consist of observations of the father's interactions with family members, an interview with the father concerning his involvement in childrearing and his description of an event that captures, for him, the "essence of fathering", and a psychiatric interview. Do fathers take on a special role when mothers are ill? Does a well father with good relationships within the family significantly reduce the risks for the children? Conceptualizations of rearing considering both parents will be developed.

Project Description:

Reflecting as much the culture as biology, psychiatric and psychological theories of development have dealt almost exclusively with mothers as the determinants of the healthy or pathological development of offspring. The father's role has been ignored or has at best been considered in its absence. Even if pathology (e.g. depression) exists in the father, mothers' behavior remains the focus in offspring outcomes. For example, in such instances concurrent depression in the mother is often ascribed to "assortative mating"; thereby ignoring any dynamic influences of the father within the family affecting either the mother's or the child's mental health. In the present study, fathers' involvement in childrearing is the focus in families with and without maternal psychopathology. The general research paradigm described in MH 02144 is utilized. The family (father, mother, two children ages 2-5 & 8-11) is brought into a home-like environment recreated in the laboratory; they are asked to participate in planned situations representative of typical day-to-day family events. Over a half day session, the family unit experiences a variety of recombinations to allow observation of the siblings alone, father alone with both children and alone with each child, and periods with the whole family together, including a family meal time. Additionally, the father is interviewed about his involvement in childrearing and asked to describe an event with the children that captures for him, the "essences of fathering". The father is also given a psychiatric assessment. These data provide opportunities to study the nature of fathers' contributions to the rearing of children and to investigate specifically questions such as: In what manner and around what issues does the father interact with the children? In what ways does paternal interaction differ with gender and age of child? Does the presence of the mother affect the paternal interaction? Do fathers take on a special rearing role when mothers are ill? Does a well father with good relationships within the family significantly reduce the risks of behavior disorders in the children? Conceptualizations of rearing considering both parents will be developed.

Significance to Biomedical Research:

This project provides data that will allow systematic study of the impact of the parenting unit on child behavior. Of particular interest will be the behavior of fathers with wives suffering from affective disorders. The father's inclusion extends information regarding protective and risk factors in the child's rearing environment.

Proposed Course:

Data collection has recently begun. About a 12 month period of collection will be required to observe and interview approximately 100 families.

Publications:

None

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02172-01 LDP

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mothers As Mediators of Cognitive Development

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI : Sarah L. Friedman Research Psychologist LDP/NIMH

Other: Tracy Sherman Guest Researcher LDP/NIMH
Marian Radke-Yarrow Chief LDP/NIMH

COOPERATING UNITS (if any)

NONE

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland

TOTAL MAN-YEARS: Person Years PROFESSIONAL:

OTHER:

.90

.85

.05

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

It is hypothesized that the mental health of the mother has profound influences on her children's cognitive growth. More specifically, symptoms associated with depression such as loss of interest and pleasure in usual activities, feelings of worthlessness and self reproach, diminished ability to concentrate, irritability, and (in mania) flights of ideas all influence the mother's functioning in ways that would be expected to interfere with her ability to transmit information and thinking skills to her child. The research subjects are mothers and children participating in the Child Rearing Study. These include 30 mothers diagnosed as normal and 58 diagnosed as clinically depressed. Segments of mother-child interactions are selected from nine hours video-taped sessions in a home-like environment. These interactions are coded so as to describe (a) the extent to which mothers impart important cognitive contents to their children and (b) the methods that mothers use in order to impart cognitive contents to their children. Cognitive contents include facts about physical, social and emotional events, methods for knowledge acquisition, representation and use, and thinking and problem-solving skills. From the description of the differences between the normal and depressed mothers' teaching behaviors and from assessments of the child's responses, it will be possible to begin isolating those aspects of the mother's behavior which place the child at risk for impaired cognitive functioning. It will also be possible to determine aspects of the mother's behavior which are associated with the development of depression in the child.

Project Description:

The major purpose of this research is to investigate mothers' influences on the cognitive development of their children.

It is hypothesized that clinical depression interferes with the mother's ability to serve as a mediator for her child's cognitive development. Symptoms associated with depression such as loss of interest and pleasure in usual activities, feelings of worthlessness and self-reproach, diminished ability to concentrate, irritability, and (in mania) flights of ideas all influence the mother's functioning in ways that would be expected to interfere with her ability to transmit information and thinking skills to her child. The hypothesis that qualities of mothering have profound influences on children's cognitive growth is supported by a large body of research. For example, being born into a lower socio-economic class limits the child's chances of improving performance on intelligence measures between the ages of 8 months and 4 years. Similar results hold when the effect of genotype on cognitive development is experimentally partialled out, as in adoption studies. These studies indicate that the environment in itself has a substantial influence on the ability of children to do well cognitively. The existing research is largely correlational, however, and does not reveal the mechanisms underlying environmentally caused interference with or enhancement of the child's mental development.

The objectives of this project are to identify (a) the extent to which mothers impart to their children important cognitive contents such as facts about physical, social and emotional events, methods for knowledge acquisition, representation and use, and thinking and problem solving skills; and (b) the methods that mothers use in order to impart cognitive contents to their children. Such methods include creating a shared focus of attention, regulating the child's attention, modeling cognitive strategies, and talking about cognitive contents. Normal mothers and mothers with psychopathology are compared. Also, mothers whose depression has been brought under medical control are examined.

The subjects are mothers and children participating in the Child Rearing Study. The sample includes 30 mothers diagnosed (SADS) as normal and 58 diagnosed as clinically depressed. The latter group includes sub-categories of minor depression, major depression, and bipolar depression. Nine hours of interaction between the mother and her two to four year old child are obtained (see Z01 MH 02144). In the last three hours an older sibling is also present.

From the video-taped sessions two types of segments of mother-child interaction are selected: interactions over a variety of rearing situations in which no special task is assigned the mother, and interactions when the mother has been given a specific teaching task.

Significance to Biomedical Research:

The findings of the study will have implications for the study of normal and deviant processes in child development. From the identification of the differences between the learning environments provided by the normal and depressed mothers, and from assessments of the child's responses, it will be possible to begin isolating those aspects of the mother's behavior which place the child at risk for impaired cognitive functioning. It will also be possible to determine aspects of the mother's behavior which are associated with the development of depression in the child.

Proposed Course:

This research has required the development of an elaborate coding system. This has been accomplished and coding has recently begun.

Publications:

Friedman, S.L. and Cocking, R.R.: Instructional influences on cognition and on the brain. In Friedman, S.L., Klivington, K.A. and Peterson, R.W. (Eds.): The Brain, Cognition and Education. New York, Academic Press, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02173-01 LDP

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Development of Sex Identity in Children

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Tracy Sherman	Guest Worker	LDP/NIMH
Other:	Grazyna Kochanska	Guest Worker	LDP/NIMH
	Marian Radke-Yarrow	Chief	LDP/NIMH

COOPERATING UNITS (if any)

NONE

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland

TOTAL MAN-YEARS Person Years

.25

PROFESSIONAL:

.20

OTHER:

.05

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This study addresses the development of young children's sex identity and knowledge of culturally prescribed sex roles, and the process by which maternal childrearing behaviors contribute to this development. Previous research has demonstrated that by the time children are 5-6 years of age, consistent sex differences appear in children's choices of activities, their motives when engaged in an activity, and their performance styles. There are few detailed data on factors contributing to these differences. The goal of this study is to fill this gap. Maternal behaviors with girls and boys are being observed over a range of activities.

This study is a by-product of the laboratory's Rearing Study which provides extensive interactional data on 100 mothers and their children, and thereby permits a focus on sex of child as a determinant of rearing environment.

The data sources are videotaped observations of mothers and their 2- 3 1/2-year-old children in a variety of naturalistic settings. Similar observations are obtained two years later. In the follow-up observations, a series of gender related probes have been incorporated which will provide information about the mother's conscious preferences for her child as well as the child's own preferences for her/himself and knowledge of the cultural sex stereotypes.

Project Description:

This study addresses the development of young children's sex identity and knowledge of culturally prescribed sex roles, and the process by which maternal childrearing behaviors contribute to this development. Previous research has demonstrated that by the time children are 5-6 years of age, consistent sex differences appear in children's choices of activities, their motives when engaged in an activity, and their performance styles. There are few detailed data on factors contributing to these differences. The goal of this study is to fill this gap. Maternal behaviors with girls and boys are being observed over a range of activities.

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The data sources are videotaped observations of mothers and their 2- 3 1/2-year-old children in a variety of naturalistic settings. Similar observations are obtained two years later. In the follow-up observations, a series of gender related probes have been incorporated which will provide information about the mother's conscious preferences for her child as well as the child's own preferences for her/himself and knowledge of the cultural sex stereotypes.

Significance to Biomedical Research:

The successful development of the child's sex identity is crucial for his/her mental health. The findings from these analyses will be examined for evidence concerning the course of sex role development and the areas of confusion and conflict for each sex.

Proposed Course:

Videotaped data have been collected on approximately 100 families. Data collection on the follow-up is underway and will continue this year. A coding scheme is being constructed which will be applied to both sets of data.

Publications:

None

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02174-01 LDP

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Parental Beliefs Regarding the Origins of Their Children's Behavior

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI	: Grazyna Kochanska	Guest Worker	LDP/NIMH
Other:	Marian Radke-Yarrow	Chief	LDP/NIMH
	Leon J. Kuczynski	Visiting Associate	LDP/NIMH

COOPERATING UNITS (if any)

NONE

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland

TOTAL MAN-YEARS: Person Years

.45

PROFESSIONAL:

.40

OTHER:

.05

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The structure of mothers' belief systems regarding the development of their children is investigated in families with and without parental psychopathology. The emphasis is on mothers' causal thinking about the factors involved in the origins of their children's behavior or personality characteristics. Affectively ill persons tend to experience helplessness and lack of personal control over life events, and manifest distorted patterns of perceived control, exaggerating responsibility for bad events and denying it regarding good events. The way mothers perceive their own causal input in the development of their children as compared to other causal factors (genetics, father's input, external events, etc.) may be a crucial influence on their rearing practices. This issue becomes one of a particular importance when clinically depressed mothers are concerned. Their possible feelings of helplessness regarding the development of their children, particularly their beliefs about their children's vulnerability to affective disorders, may influence their rearing behavior and the expectations conveyed to their children. Beliefs of 200 normal and depressed mothers are assessed with the use of a newly developed diagnostic procedure. Their beliefs will be related to their clinical diagnoses, rearing methods, and children's personality characteristics.

Project Description:

The structure of mothers' belief systems regarding the development of their children is investigated in families with and without parental psychopathology. The emphasis is on mothers' causal thinking about the factors involved in the origins of their children's behavior or personality characteristics. How people perceive causes of events, particularly how much personal control they feel they have over their lives has been widely investigated in clinical and non-clinical groups. Beliefs and feelings of helplessness have been shown to be associated with depression. Beliefs regarding personal control have not been examined in relation to mothers' perceptions of, and efficiency of, their parenting.

Conceptions of the causes or determinants of child behavior are especially relevant in studies of rearing by mothers who are clinically depressed. The mother's perception of the child's vulnerability to depression and her own feeling of control over child's development may well influence the rearing processes. Clinical and anecdotal examples in which parents have been given "explanations" or predictions concerning the likely mental health outcomes for their children demonstrate some very direct consequences of parents' beliefs for how they care for the child and for the kinds of expectations they convey to the child concerning his/her own self and future. The affectively-ill parents who believe that nothing they do really matters, that their child's mental health fate is a "given", approach child rearing differently from parents with a less deterministic and simplistic outlook.

An instrument has been developed to assess the structure of mothers' causal thinking about the origin of important aspects of children's behavior and personality. For each child attribute, the mother is asked to indicate her beliefs as to the contributions of her own intentional care-giving, personality factors in herself, father's behavior, genetics, other factors beyond her control. Mothers also evaluate the relative importance of the various child behavior areas and their satisfaction with the development of their children.

Aspects of mothers' cognitive system will be related to the characteristics of their childrearing behaviors, the characteristics of their children's personality, and to their own clinical diagnoses.

The sample is 200 normal and affectively ill mothers of preschool and school age children. The subjects differ in their clinical characteristics (minor and major depression, bipolar illness, no diagnosis), and socioeconomic status. Data on the parents' interactions with the children are available from the Rearing Study (MH 02144).

Significance to Biomedical Research:

Patterns of perceived personal control over life events have been found to be very different in normal and affectively ill groups. Depressed patients may manifest helplessness and experience lack of personal control over own lives or may feel unduly responsible for their failures. These maladaptive patterns of perceived personal control may have consequences in the domain of

their childrearing. Preventive measures and interventions will be better guided with an understanding of the interrelations of parental beliefs, their functioning with regard to their offspring, and their child's behavior.

Proposed Course:

A procedure for studying parental belief system has been developed and tested and data collection has begun.

Publications:

None

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02175-01 LDP

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Maternal Attributions and Child's Concept of Self

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI : Marian Radke-Yarrow Chief LDP/NIMH

Other: Ruth Wylie Guest Researcher LDP/NIMH

COOPERATING UNITS (if any)

NONE

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland

TOTAL MAN-YEARS Person Year PROFESSIONAL:

.20

.15

OTHER:

.05

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard un-reduced type. Do not exceed the space provided.)

The young child's cognitions and feelings about the self, how they develop, and the contributions made by specific rearing experiences are a poorly understood area of development. Here the verbalizations that mothers direct to their young children are investigated as a possibly contributing factor to the child's self-conception. Maternal attributions by normal and depressed mothers are compared. Hopelessness, helplessness, and negative feelings of self-worth are associated with adult depression. Do attributions to the child by depressed mothers contribute to similar negative self-images by the child? Children's self-concepts associated with various maternal inputs are examined. The families studied are of middle and upper-middle class backgrounds, with mothers diagnosed as normal (N = 20) or depressed (N = 20). Written transcripts of the verbal input by mother and child in ordinary rearing situations provide the primary data (MH 02144). An hour of behavior is selected to sample a variety of conditions in the interactions of parent and child. Recorded also are the behavioral context of the verbalizations, tone of voice, facial expression, and body movements. Measures of the child's self-concept and feelings are also from observational data on dependency behaviors, self-representations in play and drawings, and revelations of self in psychiatric assessments and in interactions with mother and others.

Project Description:

The young child's cognitions and feelings about the self, how they develop, and the contributions made by specific rearing experiences are a poorly understood area of development. Here the verbalizations that mothers direct to their young children are investigated as a possibly contributing factor to the child's self-conception. Maternal attributions by normal and depressed mothers are compared. Hopelessness, helplessness, and negative feelings of self-worth are associated with adult depression. Do attributions to the child by depressed mothers contribute to similar negative self-images by the child? What is the content of mothers' attributions to their 2- to 3-year-olds? How explicitly or implicitly are the messages conveyed? What are the occasions for the attributions? Children's self-concepts associated with various maternal inputs are examined. The families studied are of middle and upper-middle class backgrounds, with mothers diagnosed as normal (N = 20) or depressed (N = 20). Written transcripts of the verbal input by mother and child in ordinary rearing situations provide the primary data (MH 02144). An hour of behavior is selected to sample a variety of conditions and demands in the interactions of parent and child. Recorded also are the behavioral context of the verbalizations, tone of voice, facial expression, and body movements. Measures of the child's selfconcept and feelings are also from observational data on dependency behaviors, self-representations in play and drawings, and revelations of self in psychiatric assessments and in interactions with mothers and others.

Early findings indicate that a minority of maternal attributions contain lexical words that label the child's inner states. Most of the attributions fall into categories of child's competencies, cognitions, and ability to make decisions. Most maternal utterances carry implicit attributes, many of which are positive or negative in evaluation of the child. Until coding is completed, the "blind" on maternal diagnoses will not be broken; hence, comparisons of the two groups of mother-child pairs can not be made until then.

Significance to Biomedical Research:

The child very early acquires a sense of self and a sense of the causes of events affecting him/her personally. This cognitive underpinning has significance for subsequent development. Data from this research will provide insights into familial factors that contribute risk or protection in the developmental process, and will thereby add to knowledge that can be utilized in preventive and corrective treatment of parents and children.

Proposed Course:

Transcriptions have been completed and coding of mothers' input is well underway. Codes for child's speech and measures of the child's self concept derived from the observational data are being developed. The coding scheme for the analyses of maternal verbalizations is a very carefully elaborated system. Its rationale and preliminary finding will be presented at The International Conference on Self and Identity, Cardiff, Wales, July 8-13, 1984.

Publications:

None

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02207-01 LDP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Affective Rearing Environment: A Comparison of Normal and Depressed Parents

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Marian Radke-Yarrow	Chief	LDP/NIMH
Other:	Leon Kuczynski	Visiting Associate	LDP/NIMH
	Carolyn Zahn-Waxler	Research Psychologist	LDP/NIMH
	Karen Capolvitz Barrett	Guest Worker	LDP/NIMH

COOPERATING UNITS (if any)

NONE

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland

TOTAL ~~MAN-YEARS~~ Person Years PROFESSIONAL:

.30

.25

OTHER:

.05

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In the program of research on normal and psychopathological childrearing and child development, the affective quality of the caregiving environment is a core focus. The environment has been conceptualized in terms of the kinds and intensities of emotions and moods that characterize the caregiver and enter into the relationship between parent and child. Mothers without psychiatric diagnosis and depressed (bipolar and unipolar) mothers and their young children (15 to 24 months) and sibling (5 to 8 years) are studied. The daily pattern or profile of emotions expressed by parent and child is obtained in a minute-by-minute rating of the predominant emotion. Records are of 3 hours of interaction on 3 days. These ratings permit assessment of the pervasiveness of given emotions, the variety and lability of emotion, the variation of emotions in relation to situational characteristics, psychiatric diagnosis of mother, and characteristics of child, and the functional significance of emotions in the mother-child dyad. Another set of analyses focuses on the socialization of the child's emotions: (a) the socialization of affection and (b) mothers' strategies for handling children's emotional distress. These studies are now in coding stages.

Project Description:

In the program of research on normal and psychopathological childrearing and child development, the affective quality of the caregiving environment is a core focus. The environment has been conceptualized in terms of the kinds and intensities of emotions and moods that characterize the caregiver and enter into the relationship between parent and child. Mothers without psychiatric diagnosis, and depressed (bipolar and unipolar) mothers and their young children (15 to 24 months) and sibling (5 to 8 years) are studied.

Affective relationships of mother and child have long interested researchers of many persuasions, and have generally been dealt with in global terms of nurturance, warmth, affection and the inverse, hostility and rejection. These formulations are essentially in terms of the feelings of mother toward child. Affective dimensions of rearing can be viewed from other perspectives as well, namely, in terms of the specific forms and functions of emotional expression by the parent, and in terms of the parent's socialization of the child's expression, use, and control of emotions. These several perspectives are utilized in analyses of the observational data of MH 02144.

The daily pattern or profile of emotions expressed by parent and child is obtained in a minute-by-minute rating of the predominant emotion. Records are of 3 hours of interaction on 3 days. These ratings permit assessment of the pervasiveness of given emotions, the variety and lability of emotion, the variation of emotions in relation to situational characteristics, psychiatric diagnosis of mother, and characteristics of child, and the functional significance of emotions in the mother-child dyad.

Another set of analyses focuses on the socialization of the child's emotions: (a) the socialization of affection. Interactions involving physical contact and affection between mother and child are analyzed for the forms and functions of affection and the child's role in eliciting, reciprocating, or resisting affection and (b) mothers' strategies for dealing with children's emotional distress. The mothers' attempts to regulate and control the child's emotions in the face of distress are observed in naturalistic settings and in an experimental situation which introduces some stress; namely, the child is given an anthropometric examination by a male in a white coat.

The same dimensions of affect are measured again in the follow-up phase of the research (2 years later).

Significance to Biomedical Research:

In families with affectively ill and with normal parents, the emotional aspects of the environment are assumed to influence the child's development. These studies should provide information that translates parental depression into concrete affective rearing behaviors and identifies particularly pathogenic kinds of learning conditions for the child. The study further identifies, within a normal population, variations in rearing practices with respect to the child's emotional expression and control that will be investigated with regard to adaptive and maladaptive outcomes in the child.

Proposed Course:

These studies have required extensive code development and coding time. Coding of the first data set is now near completion. Analyses have begun and reports will follow.

Publications:

Radke-Yarrow, M. and Zahn-Waxler, C.: Familial factors in the development of socially valued behavior. In Block, J., Olweus, D.D., and Radke-Yarrow, M. (Eds.): Aggression and Socially Valued Behavior: Biological and Cultural Perspectives. New York, Academic Press, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02208-01 LDP

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Childrearing Patterns of Affectively Disturbed, Abusive, and Normal Mothers

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI :	Elizabeth J. Susman	Senior Staff Fellow	LDP/NIMH
Other:	Barbara Hollenbeck	Social Science Analyst	LDP/NIMH
	Ronald Iannotti	Research Psychologist	LDP/NIMH
	Penelope K. Trickett	Senior Staff Fellow	LDP/NIMH
	Carolyn Zahn-Waxler	Research Psychologist	LDP/NIMH
	Marian Radke-Yarrow	Chief	LDP/NIMH

COOPERATING UNITS (if any)

NONE

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland

TOTAL MAN-YEARS Person Year PROFESSIONAL:

.50

.45

OTHER:

.05

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Childrearing attitudes and reported practices in depressed, abusive, and normal mothers are investigated. Participants are mothers in two studies in the Laboratory--child rearing in families with and without parental depression (MH 02144) and childrearing in an environment in which there is parental abuse of the child (MH 02158). Mothers selected from the first study have diagnoses of unipolar depression or normal; mothers in the research on child abuse were referrals from protective service agencies and mothers from the community with no history of abuse and matched in socio-economic status. Participants completed an inventory probing childrearing attitudes, values, behaviors, and goals (Block Q-Sort). Depressed mothers were more inconsistent than normal mothers in their reported practices. They were more likely to forget promises they had made to their children and threaten punishment more often than they actually carry it out. Abusive mothers were characterized by wide-ranging and pronounced deficits in rearing. They valued harsh discipline, felt angered and disappointed by their child, devalued their child's opinion, and were critical of their child's shortcomings. Findings of this study identify specific areas in childrearing in which parents with emotional disturbances manifest deficits which may contribute to the development of emotional problems in their children.

Project Description:

The objective of this study is a comparison of the childrearing attitudes and reported practices of depressed mothers, abusive mothers, and normal mothers. The childrearing patterns of parents are thought to influence many aspects of the emotional development of their children. While much is known about the attitudes and practices of presumably normal parents, little is known about the patterns of psychiatrically disturbed and abusive parents. The higher incidence of psychiatric disorder in children of parents with affective disorders compared to children without disorders may be partially accounted for by childrearing attitudes and practices. Similarly, the emotional problems of abused children may stem from the harsh disciplinary attitudes and practices of their parents.

Methods and Findings:

Participants are mothers in two studies in the Laboratory--childrearing in families with and without parental depression (MH 02144) and childrearing in an environment in which there is parental abuse of the child (MH 02158). Mothers selected from the first study have diagnoses of unipolar depression or normal; mothers in the research on child abuse were referrals from protective agencies and mothers from the community with no history of abuse and matched in socio-economic status. Mothers completed an inventory (Block Q-Sort), probing childrearing attitudes, values, behaviors, and goals.

Findings show that abusive mothers, and to a lesser degree, depressed mothers, differ from normal mothers on a variety of childrearing attitudes and practices. Abusive mothers reported wide-ranging, pronounced, and pervasive deficits in rearing: e.g., more than other mothers they valued harsh discipline, felt angered and disappointed by their children, showed deficits on emotional expression and regulation, lacked the capacity for providing enriching or stimulating experiences, devalued the child's opinion, and interfered with the child's autonomy. These findings disconfirm previous research reports suggesting that most interactions in abuse families are positive and that problems are confined largely to incidents requiring discipline. Mothers with major depression more often than normal mothers reported use of guilt-induction techniques and high levels of protectiveness. Mothers with current major or current minor depression were more inconsistent than normal mothers in their childrearing attitudes and practices. The inconsistency appeared to reflect carryover of depressive symptoms such as memory problems and helplessness into the arena of childrearing, e.g. the mothers forgot promises they had made to their children or threatened punishment but did not carry through. Mothers with diagnoses of past depression were similar to normal mothers on a majority of their childrearing attitudes and practices, suggesting that many of the rearing problems of depressed mothers might be viewed as state-rather than trait-related.

Disturbances and deviations in parental rearing practices were more likely to be directed toward girls than boys: Mothers reported more worry, more protective efforts, less enjoyment of the parental role, less open expression of emotion and less encouragement of openness to new experiences in relation to girls than to boys. This tendency was most pronounced for the mothers who themselves experienced emotional problems. Hence girls from these families may be particularly at risk for emotional disorders. The parenting problems reported by mothers of girls reflect environmental factors that may predispose girls toward feelings of helplessness, hopelessness, and lack of self-worth, which, may be precursors of adult depression,--the incidence of which is two to three times higher in women than in men. Despite marked cultural changes in the past decade, attitudes and values regarding the socialization of boys and girls, based on the Q-sort data, seem to have remained much the same.

Significance to Biomedical Research:

Findings of this study identify specific areas in childrearing in which parents with different kinds of emotional disturbance manifest deficits. These findings may have implications for the development of strategies for therapeutic interventions for mothers of young children.

Proposed Course:

The data have been collected and analyzed and a manuscript has been submitted for publication.

This is a final report.

Publications:

None

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02209-01 LDP

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Attachment and Maternal Psychopathology

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI	:	E. Mark Cummings	Staff Fellow	LDP/NIMH
Other:		Marian Radke-Yarrow	Chief	LDP/NIMH
		Michael Chapman	Research Psychologist	Max-Planck Institut
		Leon Kuczynski	Visiting Associate	LDP/NIMH

COOPERATING UNITS (if any)

Max Planck Institute, Berlin, West Germany

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland

TOTAL MAN-YEARS

.50

PROFESSIONAL:

.45

OTHER:

.05

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The quality of the child's emotional bond or attachment to the mother has been shown to be an important predictor of the child's emotional and social functioning in early childhood. The present research focuses on the nature of attachment relationships in children whose mothers are clinically depressed compared with children of normal mothers. The Ainsworth paradigm was used to assess attachment. Insecure attachment was found to be significantly more frequent in offspring of parents with a major affective disorder, with the highest rate in children of bipolar parents.

Project Description:

The quality of the emotional bond or attachment to the mother appears to be one of the most important influences on the infants' general emotional and social functioning, and a powerful predictor of later development. Children with poor quality or insecure attachments are less effective in exploring their environments, are generally more fearful and alienated, are less competent with peers, and are at greater risk for the development of behavior problems. Of the many possible factors within the mothers that may interfere with the establishment of a secure relationship with her young child are the mother's affective state and affective attributes. The present research focuses on the quality of the child's attachment to mother in relation to the affective status of the mother. Offspring of parents with affective disorders have been shown to be at greater risk for the development of affective disorders themselves. These parents may be less able to interact effectively with their children and to establish good relationships, hence their children may be less well equipped in the domains of social and emotional development.

Families in the Rearing Study (MH 02144) are the research participants. The Ainsworth Strange Situation Paradigm was used to assess the attachment relationship. Affective qualities of the mothers were assessed in a number of ways: (a) SADS diagnosis of normal or depressed (bipolar, major unipolar, minor depressed); (b) severity of depressive episodes; (c) daily self-ratings of moods; (d) expressed emotion in rearing behavior (a minute-by-minute coding of affect in a 9-hour behavior sample).

Fourteen offspring (15 to 47 months of age) of bipolar mothers, 42 offspring of mothers with major depression, 12 children of mothers with minor depressive disorders, and 31 children of mothers with no history of affective illness were studied. In general, children of mothers with a major affective disorder had a higher incidence of insecure attachment than controls but the bipolar group had an even higher occurrence of insecure attachment than the major depressive group. In addition, there was a significantly higher incidence of "very deviant" insecure attachments (children who were insecure on multiple dimensions) in children of mothers with major affective disorders. Children of minor depressive mothers and normal mothers did not differ.

Significance to Biomedical Research:

These data help to identify processes within families in which there is maternal depression. They contribute information which is needed to explain the link established in epidemiological studies between parental depression and offspring disturbance. They show that, in addition to genetic risk, many of the young offspring of depressed parents experience conditions of rearing which, in themselves, are known to contribute to developmental problems, whether or not parental depression is present.

Proposed Course:

A manuscript, attachment relationships in children of depressed and normal mothers, is being prepared for publication. In continuing work (a) more detailed analyses will be made of affective aspects of parental behavior contributing to the development of secure and insecure attachments; (b) alternative indices of attachment, and attachment beyond the first years are being measured; and (c) the relations between attachment and the quality of the young child's affective and social development will be examined.

Publications:

Cummings, E.M. and Bjork, E.L.: Search behavior on multi-choice hiding tasks: Evidence for an objective conception of space in infancy. Int. J. Beh. Dev. 6: 71-87, 1983.

Cummings, E.M. and Bjork, E.L.: Perseveration and search on a five-choice visible displacement hiding task. J. Genet. Psychol. 142: 283-291, 1983.

Bjork, E.L. and Cummings, E.M.: Infant search errors: Stage of concept development or stage of memory development? Memory and Cognition 12: 1-19, 1984.

Cummings, E.M. and Beagles-Roos, J.: Toward a model of infant day care: Studies of factors influencing responding to separation in day cre. In Ainslie, R. (Ed.): Quality Variations in Day Care: Implications for Child Development, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02210-01 LDP

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Symbolic Processes in Children at Risk for Depression: The Case of Play

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI : Karen Caplovitz Barrett Guest Researcher LDP/NIMH

Other: Sarah L. Friedman Research Psychologist LDP/NIMH
 Malcolm Watson Associate Professor Brandeis Univ.
 Dennis Wolf Research Psychologist Harvard Univ.

COOPERATING UNITS (if any)

Brandeis University; Harvard University

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland

TOTAL YEARS	PERSON YEARS	PROFESSIONAL	OTHER
1.35		1.30	.05

CHECK APPROPRIATE BOX(ES)

(a) Human subjects (b) Human tissues (c) Neither

(a1) Minors

(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The importance of children's play as an avenue for development and as a central activity revealing of the child's cognitive and affective status has long been recognized by researchers and clinicians. Play is sensitive to the important developmental changes in symbolic capacities during early childhood. Moreover, play also reveals concerns that the child has. It is hypothesized that a child's experiences with an affectively disordered mother should influence both cognitive and affective features of the child's play. It is also predicted that mood disorders will interfere with the mother's ability to become involved with the child in play. This study will test these hypotheses by examining the narrative themes, the affect-relevant themes (e.g., aggression, fear, helplessness, nurturance, mastery), the cognitive level (sensorimotor, presymbolic, symbolic), the veridical affect during the play, and other more specific characteristics of the play. The mother's intervention in the child's play will also be assessed - the interventions, the strategies used to communicate the borderline between fantasy and reality, and how much departure from reality is allowed or encouraged. Participants are 2 to 4 year olds and their mothers who have been videotaped in semi-naturalistic conditions representative of early rearing experiences. Mothers have been diagnosed as normal or as depressed (bipolar, unipolar, minor depression). A comprehensive coding system has been developed for coding variables such as maternal involvement, directionality and hedonic valence of exchange between mother and child in play, narrative themes of pretense play, and cognitive levels of the mother's and child's play.

Project Description:

The importance of children's play as an avenue for development and as a central activity revealing of the child's cognitive and affective status has long been recognized by researchers and clinicians. Play reveals capacities to sustain attention and interest in objects, as well as to use knowledge, affect, and imagination in a creative way. It is sensitive to developmental changes. The child's growing abilities to recreate in action events that occurred in the past, to conceive of certain objects or behaviors as adequate representations for other objects or behaviors, and to free its thinking from stimuli in the "here and now" are evidenced in the child's play. Play is also sensitive to concerns that the child has. It is a means through which the young child, whose verbal skill is limited, expresses what is on its mind. Play, it is assumed, makes possible wish fulfillment as well as repetition and gradual "working through" of traumatic or unassimilable experiences. The patterns of children's affect while they engage in different forms and levels of play have not been studied; in fact, researchers often appear to assume that play is accompanied by joy and excitement.

It is hypothesized that a child's experiences with an affectively disordered mother should influence both cognitive and affective features of the child's play. It is also predicted that mood disorders will interfere with the mother's ability to become involved with the child in play.

This study will test these hypotheses by examining the narrative themes, the affect-relevant themes (e.g. aggression, fear, helplessness, nurturance, mastery), the cognitive level (sensorimotor, presymbolic, symbolic), the veridical affect during the play of the children, and other more specific characteristics of the play. The mother's activity with the child in play also will be assessed: the supportive or interfering nature of the mother's interventions, the strategies used to communicate the borderline between fantasy and reality, and how much departure from reality is allowed or encouraged.

The research participants are 2 to 4 year olds and their mothers, who have been seen in semi-naturalistic conditions representative of early rearing experiences (see MH 02144). Mothers have been diagnosed as normal or as depressed (bipolar, unipolar, minor depression).

Data for the present study are coded from videotapes. A comprehensive coding system for scoring the videotapes has been developed. This includes assessment of maternal involvement, directionality and hedonic valence of exchange between mother and child in play, and the narrative themes of pretense play and the cognitive levels of the mother's and the child's play. The tapes are coded by researchers who are blind to maternal diagnosis.

Significance to Biomedical Research:

This study concerns the impact of maternal depression on some of the most important processes of development during the toddler and preschool period. Findings will have important implications for clinicians and parents concerned with how the behaviors of depressed mothers (e.g., the emotions they express, how they intervene in their children's activity) affect their child's psychological status.

Proposed Course:

Data coding has begun. Data analyses will be under way in the coming year.

Publications:

Campos, J., Barrett, K.C., Lamb, M., Goldsmith, H., and Stenberg, C.: Socioemotional development. In Haith, M. and Campos, J. (Eds.): Handbook of Child Psychology: Infancy and Developmental Psychobiology. New York, John Wiley & Sons, 1983, pp. 783-916.

Campos, J. and Barrett, K.C.: Toward a theory of emotional development. In Izard, C., Kagan, J., and Zajonc, R. (Eds.): Emotion, Cognition, and Behavior. New York, Cambridge University Press, 1984, pp. 229-262.

Caplovitz, K.C., and Campos, J.: Emotion and self. In Harre, R. and Lamb, R. (Eds.): Encyclopedic Dictionary of Psychology. Oxford, England, Basil Blackwell Publishers, Inc., in press.

Bertenthal, B., Campos, J., and Barrett, K.C.: Self-produced locomotion: An organizer of emotional, cognitive, and social development in infancy. In Emde, R. and Harmon, R. (Eds.): Continuities and Discontinuities in Development. New York, Plenum Press, in press.

Campos, J., Emde, R., and Caplovitz, K.: Emotional development. In Harre, R. and Lamb, R. (Eds.): Encyclopedic Dictionary of Psychology. Oxford, England, Basil Blackwell Publishers, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00478-28 LN

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neural mechanisms of memory and habit formation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	M. Mishkin	Chief	LN NIMH
Others:	E.A. Murray	Senior Staff Fellow	LN NIMH
	J. Bachevalier	Visiting Associate	LN NIMH
	D. Kowalska	Visiting Associate	LN NIMH
	H. Petri	Chairman	Towson State Univ.
	W. Overman, Jr.	Asst. Professor	Univ. of North Carolina

COOPERATING UNITS (if any)

Towson State University
University of North Carolina

LAB/BRANCH

Laboratory of Neuropsychology

SECTION

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:

6.5

PROFESSIONAL:

3.0

OTHER:

3.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The anterior temporo-insular cortex in the macaque consists of the highest-order sensory processing stations for all the sensory modalities. We have proposed that this cortex contains the stored representations of stimuli to which the organism has been exposed. The storage is presumed to be the result of activation by anterior temporo-insular neurons of a limbo-thalamo-cortical pathway, which actually consists of two parallel pathways, one involving the amygdala, the magnocellular portion of nucleus medialis dorsalis, and orbital frontal cortex, and the other involving the hippocampus, anterior thalamic nuclei, and the anterior cingulate cortex. Recognition memory occurs when the stored representation of a past stimulus is reactivated by a current stimulus, and associative memory occurs when that stored representation is linked to the stored representation of another stimulus or another event, such as a location, an emotion, or a motor act. Evidence has been obtained suggesting that the amygdaloid circuit is selectively involved in the first of these functions (i.e. stimulus-stimulus associations), whereas the hippocampal circuit is selectively involved in the second (i.e. stimulus-location associations). All of these forms of memory can be distinguished from habits, which appear to be independent of the limbo-thalamic system and may depend instead on the cortico-striatal system.

PROJECT DESCRIPTION:

The objective of the studies in this project is to delineate the neural system underlying memory formation in the monkey and to differentiate it from the neural system that underlies habit formation. The methods used include behavioral analyses of the effects of selective cerebral ablations and disconnections combined with anatomical analyses of functional neural pathways. The rationale and design of the studies are often based directly on information derived from other projects in this laboratory, many of which deal with the pathways for, and mechanisms of, stimulus processing and encoding. The results from these other projects suggest that the sensory system for each modality is composed of two hierarchically organized corticocortical pathways, one directed ventrally to the temporal-lobe limbic system and concerned with object perception, the other directed dorsally to the frontal-lobe motor system and concerned with spatial perception. The ultimate goal of this project is to determine how object and spatial perceptions in the different modalities are formed into memories, how these different memories are associated with each other, how they evoke emotions and motor acts, and how they lead not only to these cognitive events but also to habit formation. Our progress in understanding each of these processes will be described in turn.

(1) Recognition memory

Previous studies suggested that one-trial object recognition (delayed nonmatching-to-sample with trial-unique objects) depends on a reciprocal cortico-limbo-thalamic pathway that leads to the storage of the encoded representation of the stimulus in anterior temporo-insular cortex. New studies have shown that this pathway actually contains two relatively independent limbo-thalamic segments, one from the amygdala through the amygdalofugal pathways (AFP) to the magnocellular portion of n. medialis dorsalis (MDmc), and the other from the hippocampus through the fornix (Fx) to the anterior thalamic nuclei (Ant N). The evidence is based on comparison of the effects of separate and combined AFP and Fx transections, as well as of separate and combined MDmc and Ant N ablations. In both cases, the combined lesions yielded significantly greater recognition losses than did the separate lesions. These recent results resemble our original finding that combined removal of the amygdaloid complex (A) and hippocampal formation (H) yielded far greater recognition losses than did their separate removal. In view of the importance of the two limbo-thalamic pathways for recognition memory, we have initiated a series of studies to determine whether their prefrontal targets might also be involved in this function. The results of our first study indicated that recognition memory was severely impaired following ablation of ventromedial prefrontal cortex (VM), which contains the projection areas of both MDmc and Ant N. In contrast, recognition memory was essentially unaffected by lesions of the dorsolateral prefrontal cortex (DL), an area to which the parvocellular division of MD projects. In a second study, we prepared monkeys with lesions of the inferior prefrontal convexity (IC), another projection target of the parvocellular division of MD, but one that is known to have a pervasive influence on learning ability in the monkey. Despite their marked difficulty in relearning the recognition task, however, the monkeys with IC lesions were unimpaired when delays and lists were

lengthened, their average performance being equivalent to that of animals with DL lesions. Comparison of these data with those we obtained many years earlier on prefrontal function suggests that: (i) presumably because of its connections with the limbo-thalamic pathways, ventromedial prefrontal cortex (about which there has been little functional information until now) is far more important than either the dorsolateral or inferior prefrontal cortex for general memory processes; (ii) the classical delayed-response deficit after dorsolateral prefrontal lesions may represent a perceptual-mnemonic impairment in spatial functions rather than a strictly mnemonic one; and (iii) the perseverative tendencies produced by lesions of the inferior prefrontal convexity are the cause of their impairment in learning and not the consequence of any memory loss.

Further investigation of the functional significance of the ventromedial prefrontal cortex have become important goals of our research. For example, will damage to the orbital cortex alone (the target of MDmc) or of the adjacent anterior cingulate cortex alone (the target of Ant N) be sufficient to produce the severe memory loss reported above, or is their combined damage necessary, as was found in all of our previous investigations of the amygdaloid and hippocampal systems? As a first step toward answering this question, we are attempting by anatomical study to divide medial prefrontal cortex more precisely than before into two zones - one related predominantly to MDmc and the other to Ant N. Preliminary results indicate that, following an HRP injection into subcallosal prefrontal cortex, both anterograde and retrograde label are found mainly in the dorsal part of MDmc, extending along its entire length; anterograde label is also present, however, in nuclei parataenialis, centralis densocellularis, and reuniens. An HRP injection in the anterior cingulate cortex, in front and just above the genu of corpus callosum, yielded anterograde and retrograde label in the nucleus anterior medialis as well as in the intralaminar and midline nuclei, including centralis densocellularis, centralis laterocellularis, paraventralis, and reuniens. These findings, which are in general agreement with conclusions derived previously from evidence on retrograde cell loss after lesions of the medial prefrontal cortex, indicate clearly that the subcallosal portion of prefrontal cortex is interconnected with MDmc, whereas the precallosal and supracallosal portions are predominantly interconnected with Ant N. While the anatomical organization of the medial thalamic projection to prefrontal cortex will be investigated further, sufficient evidence has now been obtained to initiate the partial lesion study.

As indicated above, all of our experimental evidence to date demonstrates that severe deficits in recognition memory, a hallmark of global amnesia in man, can be induced in monkeys only by combined damage to the two limbo-thalamic segments of the memory system. Although these findings are consistent with the known neuropathology of amnesic patients with temporal lobe resections or Korsakoff's Disease, one piece of clinical evidence remains suggestive that damage to the hippocampal formation alone is sufficient to produce the amnesic syndrome. The evidence comes from patients with cerebral infarcts in the territory of the posterior cerebral artery, which is known to provide the blood supply for a major portion of the hippocampal formation but not for the amygdala. From the few clinical studies that included neuropathological

reports, however, it appears that the infarcts involve not only the hippocampus but also the inferior temporal cortex, the stria terminalis, portions of the thalamus, or combinations of these, suggesting that hippocampal damage alone may not have been responsible for the amnesia. To examine this possibility experimentally, we are attempting to correlate the behavioral and neuropathological sequelae of occluding the posterior cerebral artery in monkeys. Preliminary results from a small number of animals indicate that post-occlusion effects on visual recognition memory vary from almost no loss in some cases to severe loss in others. Neuropathological analysis of these cases will allow correlations between severity of memory disorders and extent of neural damage. The preparation promises to provide an animal model of the syndrome of permanent or transient global amnesia that follows reduction of blood supply in the territory of the posterior cerebral artery in man.

(2) Recency memory

Like their efferent pathways and thalamic targets, the amygdala and hippocampus make approximately equal contributions to recognition memory. In the case of other forms of memory, however, new results indicate that these two limbic structures make very different contributions. In one experiment, monkeys were trained preoperatively on a visual recognition task and, separately, on a tactual recognition task, with the same small set of forty objects comprising the stimuli for both modalities. One group of monkeys then received amygdalotomies and the other, hippocampectomies, after which both were retrained on the two intramodal memory tasks to a high level of performance. Unlike the previous memory studies, which revealed equally mild impairments after the two different lesions, this experiment yielded a significantly greater impairment after the amygdaloid than after the hippocampal lesions.

(3) Associative memory

The same animals described in (2) were subsequently tested for their ability to perform the recognition task across modalities, i.e. to choose between two visual stimuli after one had been presented as a tactile sample. In this case, the difference between the two groups was even greater than before. Thus, whereas the hippocampectomized monkeys continued to perform at a high level, the amygdalotomized monkeys fell to chance performance. Nearly the opposite results were obtained in a second study that tested the ability of monkeys to remember the spatial location of visual objects. In this case, monkeys given amygdalotomy were able to regain the level of performance they had achieved preoperatively, whereas those given hippocampectomy failed to rise above chance. The results of these two complementary experiments indicate that although both the amygdala and hippocampus are important for associative memory, their roles are totally different, the first apparently being critical for object-object association and the second for object-place association. Many further analyses along the lines of these two experiments will of course be necessary before the selective associative memory functions of the amygdala and hippocampus can be identified with confidence. For example, the association of an object with an affective state, such as fear,

pleasure, etc., appears to depend much more heavily on the amygdala than on the hippocampus. New support for this view is being obtained in an experiment showing that one-trial object-reward association is impaired more by amygdaloid than by hippocampal lesions (although neither deficit approaches in severity the one produced by combined removal of these two structures). By contrast, because of the contribution to spatial memory that is made by the hippocampus, the association of objects with spatially directed motor acts could depend more heavily on the hippocampus than on the amygdala. Studies to examine this possibility are being planned.

(4) Habit Formation

On all of the memory tasks described, the deficits are especially severe when removals of the amygdala and hippocampus are combined. Yet, even the combined limbic lesion does not affect all forms of learning and retention. For example, despite their rapid forgetting in one-trial object recognition, animals with the combined limbic lesions have no difficulty learning object discriminations, at least in the standard situation where trials are repeated 3-4 times per minute. In an attempt to resolve this discrepancy between rapid forgetting and successful learning, we tested whether object discrimination learning would be prevented in animals with limbic lesions if intertrial intervals exceeded the putative memory span. Surprisingly, animals with the combined amygdalo-hippocampal lesions learned to discriminate a long list of object pairs even though the list was presented only once every 24 hours. The same result was obtained in animals with severe losses in recognition memory following ventromedial prefrontal lesions. Thus, although animals with lesions of the limbic system and related structures have an extremely short memory span, they can retain and accumulate information gained from single discrimination learning trials separated by 24-hour intervals. This paradoxical success in the presence of severe memory loss implies the existence of an important retention mechanism outside the limbic structures of the temporal lobe.

We have now performed experiments to characterize further the essential difference in function between the limbic and nonlimbic retention mechanisms. Our results suggest that the limbic system is critical for high levels of retention of object-reward associations after a single acquisition trial with short lists of objects, or after two or three repetitions with long lists of objects but short intertrial intervals. With greater repetition, however, retention of object-reward associations can be mediated in the absence of the amygdala and hippocampus, and the retention appears to be independent of both list length and delay. To distinguish this form of learning and retention from memory, we have labelled it 'habit formation'. Further investigation of this mechanism of habit formation as well as elucidation of its neural substrate have become important goals of our research. For example, preliminary evidence regarding the effects of damage to inferior temporal cortex (area TE) indicates that this cortical region is important not only for the limbic memory system but also for the nonlimbic habit system.

SIGNIFICANCE TO MENTAL HEALTH RESEARCH:

In the process of investigating the role of various temporal-lobe structures in the visual memory of the monkey, we obtained a result that is particularly exciting because it appears to solve the long-standing puzzle concerning the neuropathology underlying the syndrome of global amnesia in man. This syndrome, which is characterized by a profound inability to remember new experiences, has been attributed in the clinical literature to destruction of the hippocampus. Yet, attempts to duplicate this syndrome in monkeys by removal of the hippocampus alone have largely failed. What we have found in our studies, for both recognition memory and associative memory, is that if damage to the hippocampus is combined with damage to the amygdala then a profound memory loss does ensue. The discovery has not only resolved the discrepancy between clinical and animal findings but has also provided new insight into the neural substrate of memory. Specifically, it has led to the development of a hierarchical model of recognition and associative memory involving a cortico-limbo-thalamic memory circuit that may well serve as the foundation for all cognitive processes beyond perception, including thought. As we gain further understanding of the memory system, and how it differs from the noncognitive system for habit formation, we will inevitably gain a better understanding of thought and its breakdown in normal and abnormal behavior.

PROPOSED COURSE OF RESEARCH:

Since combined removal of the prefrontal cortical targets of the magnocellular portion of n. medialis dorsalis and the anterior thalamic nuclei produced such a severe loss in visual recognition memory, we are now examining the effects on memory of damaging these two targets separately. Also, having now found severe recognition losses in both object vision and touch after lesions of the limbo-thalamic system, we are attempting to devise tests of auditory recognition and visual spatial recognition, with the aim of determining whether the system is indeed critical for recognition in all perceptual modalities. In addition, we shall continue to test our theoretical model against the effects of occluding the posterior cerebral artery. Further attempts will be made to differentiate between amygdaloid and hippocampal contributions to associative memory, and we shall test whether any distinctions found are carried further through the thalamic and prefrontal segments of the circuit. Finally, we shall initiate studies to explore the neural basis of habit formation, with the cortico-striatal projection system as our initial target.

PUBLICATIONS:

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Murray, E.A. and Mishkin, M. Combined removal of the amygdala and hippocampus in monkeys produces severe tactual as well as visual memory deficits. J. Neurosci. (in press), 1984.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02032-08 LN

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neural coding of visual stimuli in the awake monkey

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2.0

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1.0

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- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

To understand how the visual system in the monkey analyzes, classifies, and stores visual stimuli, we have been recording the responses of single neurons in two of the stations known to be critical for those functions, namely, striate cortex and inferior temporal cortex, while the animals perform behavioral tasks. We have found that attention to the stimulus to detect its dimming causes a weakened response to the stimulus by inferior temporal neurons, whereas attention to the same stimulus in order to discriminate it from another causes an increased neuronal response. Other factors, such as the particular feature of the stimulus being attended to, e.g. its texture or pattern, and the exact timing of the motor response also modify the neuronal discharge. In both inferior temporal cortex and striate cortex the pattern of a stimulus appears to be encoded not only by the number of action potentials in the neuronal discharge but also by the temporal sequence of action potentials. The temporal sequences are very similar from one neuron to the next implying that the mechanisms for producing stimulus-evoked spike trains are limited, and so encoding must involve various combinations of a few fundamental sequences.

PROJECT DESCRIPTION:

Objectives:

The primate visual system has two major functions - one is to allow orientation in and guidance through the world, and the other is to distinguish visual stimuli from each other. These two functions appear to be served by two different chains of brain regions which act as largely independent processing pathways or systems. The system for distinguishing stimuli presumably gives rise to visual perceptions and memories of stimuli. Behavioral experiments have shown that many of the critical brain regions involved in this particular system are cortical. We are studying the responses of single neurons in this system's first and last stages, the striate and inferior temporal cortices, respectively.

The investigations take two main directions. In the first we are studying how changes in task demands, particularly attentional demands, influence the neuronal responses to visual stimuli in the inferior temporal (IT) cortex.

In the second, we are trying to discover how the information in visual patterns is encoded in the neuronal discharge both in striate and inferior temporal cortex. The parameter most commonly used to measure the neuronal response to a visual stimulus is the number of action potentials, or spikes. In many records, however, there appears to be some fine structure to the stimulus-locked neuronal response, which is not well represented by the number of spikes. For example, a change in the visual pattern can cause a change in the latency of the discharge often by 30-40 milliseconds and occasionally by as long as 100 ms. Frequently, the shape of the discharge also changes from, say, a large burst of spikes in response to one stimulus to a gradual increase in the number of spikes in response to another. Such phenomena suggest the possibility that the temporal pattern of the neuronal discharge encodes the spatial pattern of the stimulus.

Major findings:

To investigate how attention to a visual stimulus modifies the IT neuronal response to that stimulus, we first insure that the stimulus is presented at a single specific retinal location. In the basic (fixation) task the monkey fixates a spot of light while its eye position is closely monitored. A stimulus is then presented in a given location. The fixation point then dims, and if the monkey makes a behavioral response to indicate it has detected the dimming, it receives a reward. The attentional demand is then varied (stimulus attention task) such that the stimulus dims instead of the fixation point, but the monkey must still respond to the dimming to receive a reward. If it responds correctly, we infer that the monkey had directed its attention to the stimulus in order to detect the change in luminance. Most cells with a response to the visual stimulus show a decreased response when the monkey performs the stimulus attention as compared with the fixation task. This result was a surprise since in other areas studied with these same behavioral tasks, such as posterior parietal cortex, stimulus attention leads to an increased or enhanced neuronal response.

In an attempt to understand this paradoxical result, we designed a new task in which stimulus attention trials (stimulus A) were intermixed with trials in which a different stimulus appeared (stimulus B). On trials with stimulus B the monkey could obtain a reward by responding to its onset (immediate response) rather than waiting for it to dim (delayed response) as it was required to do on trials with stimulus A. Many IT neurons that gave the decreased response in the stimulus attention task gave an enhanced response to the same stimulus in this stimulus discrimination task.

To investigate this phenomenon further we have introduced a discrimination task involving four stimuli composed of all combinations of two outline shapes, a square or circle, and two internal textures, stripes or dots. Either stimulus dimension may be made relevant, e.g. the reward may be made contingent on immediate response to the onset of a square and a delayed response to the onset of a circle; or indeed, any other arbitrary rule (i.e. reward contingency) can be adopted.

Preliminary results indicate that neurons will often respond differently to the very same stimulus compound depending on whether the animal is attending to one of its dimensions or another. Moreover, when the animal is attending to a given dimension, neurons may respond differently to the same stimulus compound depending on whether the animals must respond to it immediately or wait for it to dim. For some neurons the response to a given stimulus compound changes as the monkey discovers which rule is in effect. These changes in neuronal response are not always reflected simply in changes in the strength of the neuronal discharge. In some cases, a neuron may show a sustained response to a stimulus compound when one rule is in effect and show a transient response to the same stimulus compound when another rule governs. Other discharge characteristics that may change are response latency and consistency of response onset.

To investigate these and other aspects of the neuronal discharge, we have adopted a method that makes no assumptions about which parameters best characterize the spike train. There have been many other approaches to the problem of whether the shape of the spike train (the envelope, waveform, or specific sequence of interspike intervals) carries information, but most of them have concentrated on a statistical description of the neuronal discharge without attempting to correlate specific components of the spike train with the information being carried. The approach described below was designed to discover how stimulus information is encoded.

In order to correlate visual information with neuronal responses, we stimulated the neurons with patterns which, in principle, represent all possible visual features, but which are themselves unique. A set of two-dimensional visual patterns based on Walsh functions was chosen because such a set is easily produced. The complete set consists of the first 64 Walsh functions and their black-white, or contrast, reversals, making 128 patterns in all.

The patterns, each covering the receptive field of the neuron for 400 ms, were presented one per behavioral trial, while the monkeys held their eyes still.

The entire set was presented several times while the responses of a single neuron were recorded. The spike train of each individual response was converted into a spike density diagram by replacement of each spike with an appropriate probability density function, in this case a Gaussian pulse. This estimates the probability of spike occurrence. The average spike density function of all presentations of a particular pattern was used to measure the neuron's response to that pattern. The average spike density functions were then decomposed into their principal components using the Karhunen-Loeve transform. This is the linear transform which represents the response probability density waveforms with the lowest error for any given number of components. It also has the property that the coefficients are uncorrelated with each other, so that changes in one coefficient will not affect the coefficients of the other basic waveforms.

We have studied in this way 60 neurons from the inferior temporal cortex of 4 monkeys. Two of these neurons gave a response to a bar of light but no response to the stimulus set. All others responded to at least some members of the set. In the typical cell, 3-5 waveform components represented most of the energy of the neuronal responses to the patterns, with the first three waveforms being similar across virtually all of the neurons. The first waveform represents an increase followed by a slow decrease in firing during the 400 ms of stimulus presentation (tonic response). This principal component correlates highly (r of .80-.98) with the total number of spikes. The second principal component represents a transient peak of excitation lasting typically 80-100 ms followed by a slow wave of inhibition.

To determine whether the waveform components actually contain information about the patterns, we tested whether the coefficients were distributed randomly among the patterns by using the recently developed bootstrap technique. The neuron's responses to the set of patterns were taken as being representative of the underlying population of responses assuming that the responses were randomly distributed (the null hypothesis). Many new sets of responses were constructed by randomly assigning actual responses to each of the visual patterns. The average of all these response sets formed an estimate of the population under the null hypothesis. The distributions of both the spike counts and the principal components of the actual responses differed significantly ($p < 0.01$) from those of the randomly constructed responses, indicating that the response waveform of the neurons was indeed dependent on the stimulus pattern.

A plot of the coefficients of the principal components shows that responses to the patterns form a continuum, with little indication of grouping of the coefficients into separate clusters. The neurons thus seem to be conveying graded information about the stimuli on their receptive fields by using a few fundamental patterns of the spike sequence.

After finding that the waveforms of the responses were not random in IT cortex, we repeated the experiment in striate cortex, but with the patterns reduced in size to 3 degrees square. We have recorded 23 neurons from two monkeys. The pattern of results seen in IT neurons was repeated in striate neurons. Both the spike count and the first principal component once again

allowed differentiation of one visual pattern from another, and, when they did not, then the second and subsequent waveforms often allowed differentiation. The greatest differences between results from the two cortical stations were: (1) the amount of energy in the signal in the first principal component was generally higher in striate than in IT neurons; and (2) the number of patterns eliciting responses was greater in striate than in IT neurons. The latter observation suggests that the pattern space of an IT neuron is more limited than the pattern space of a striate neuron.

When the striate neurons were divided into the conventional categories of simple and complex, no differences were found in the principal components of the responses. While we had expected that simple cells would encode some information about each pattern, we were surprised to find that complex cells did so as well. We have now been able to show this result for both simple and complex cells in two ways: First, as in IT cells, the principal components and the spike counts depend upon the pattern represented; and second, the responses to a pattern and to its black-white, or contrast, reversal were often inversely related. Such a result is to be expected from models of simple-cell receptive fields which show a spatial separation of excitatory and inhibitory regions. For complex cells, however, the usual model of the receptive field is a uniformly distributed mixture of excitatory and inhibitory regions, and the prediction from such a model would therefore be an equivalence of responses to the onset of identical patterns of opposite contrast. While that was the case for some of the stimulus pairs, for others there was a stronger response to one of the pairs than to its contrast reversal, which sometimes elicited no response at all. The results thus argue against the traditional model of the receptive field of a complex cell, and support instead a model proposed recently by Dr. Spitzer in her dissertation indicating a separation of the excitatory and inhibitory regions within the receptive fields of many complex cells.

SIGNIFICANCE TO MENTAL HEALTH RESEARCH:

Many psychiatric and neurological disorders are accompanied by disordered attention, perception, and memory. The goal of this project is to gain an understanding of the mechanisms that normally underlie these basic cognitive processes. That understanding should aid in developing strategies for effective palliative treatment of cognitive deficits and for restitution of cognitive function.

PROPOSED COURSE OF RESEARCH:

The experiments dealing with the influence of attention on neuronal responses to visual stimuli will require several months to complete. We have recently received new equipment that will allow far more flexibility in the generation of visual stimuli than was available before. We will thus be able to conduct more sophisticated experiments aimed at determining the kind of visual information that is encoded in the spike train. For example, we plan to study the influence of such manipulations as changing the retinal location of a pattern, changing the size of a pattern, and adding or overlaying one pattern on another. We also intend to do some preliminary single-unit responses in

the amygdala, which is a major point of entry into the limbic system from the final visual station in the inferior temporal cortex.

PUBLICATIONS:

Richmond, B.J., Wurtz, R.H., and Sato, T. Visual response of inferior temporal neurons in the awake rhesus monkey. J. Neurophysiol. 50: 1415-1432, 1983.

NOTICE OF INTRAMURAL RESEARCH PROJECT

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Functional mapping of sensory systems

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 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Extensive cortical and subcortical areas in the rhesus monkey are involved in processing visual information. The full extent of these areas has been delineated by application of the [¹⁴C] 2-deoxyglucose method and comparison of metabolic activity in visually stimulated versus visually deafferented cerebral hemispheres. The visual-nonvisual borders of two cortical visual pathways, an occipito-temporal pathway known to be critical for object vision and an occipito-parieto pathway known to be critical for spatial vision, have been specified. In addition, their points of interaction with prearcuate and inferior prefrontal cortex and with limbic, striatal, and diencephalic structures were identified. We have also quantified the functional contribution of the forebrain commissures to vision through a comparison of monkeys prepared with complete vs partial visual deafferentation of one hemisphere (i.e. optic tract section plus forebrain commissurotomy vs. optic tract section alone, respectively). Finally, we have identified some of the cerebral areas subserving multimodal functions through a comparison of monkeys given visual stimulation only and others given visual plus somatosensory stimulation.

PROJECT DESCRIPTION:

Extensive areas of cortical tissue beyond primary visual cortex are known to be critical for higher-order visual functions. These areas appear to be organized into two main pathways: First, a ventral pathway from striate through prestriate to inferior temporal cortex, and from there to inferior prefrontal cortex; and second, a dorsal pathway from striate through prestriate to inferior parietal cortex, and from there to the prearcuate region of dorsal prefrontal cortex. Both pathways send projections in addition to various thalamic, limbic, and striatal structures. Since the full extent and precise boundaries of these cortical pathways are still undefined, as are their exact points of contact with their subcortical forebrain targets, the [^{14}C] 2-deoxyglucose method for measuring local cerebral glucose utilization (LCGU) was applied in an attempt to gain a comprehensive picture of the entire functioning visual system.

Experiment 1:

To achieve this functional map of the visual system, we prepared monkeys with a tract section combined with section of the forebrain commissures, thus visually deafferenting one hemisphere while leaving the other intact. This made it possible to compare LCGU values in a "seeing" and a "blind" hemisphere within the same animal and thereby identify and delineate the areas related to vision.

In the initial study the 2-deoxyglucose method was applied while monkeys, restrained in a primate chair, either 1) passively viewed a high-contrast geometric pattern mounted on a rotating drum that surrounded them, or 2) actively performed a visual pattern discrimination task that required a response with the hand opposite the blind hemisphere. In this task a positive stimulus was paired with one of a series of negative stimuli in sequential blocks of trials, and correct responses were reinforced with a water reward.

Both conditions of visual stimulation yielded conspicuous LCGU reductions in the "blind" right as compared with the "seeing" left hemisphere cortically, not only in the geniculostriate system, but throughout all of prestriate, inferior temporal, and inferior prefrontal cortex. The areas of depressed LCGU included tissue adjacent to the inferior temporal cortex in the upper bank of the superior temporal sulcus and in the fusiform and perirhinal areas. Subcortically in the temporal lobe, side-to-side differences were seen in lateral and basolateral amygdala, posteroventral putamen, ventral claustrum, and tail of caudate. Subcortically in the frontal lobe, hemispheric differences were seen in the anterior part of the head of the caudate nucleus. Asymmetries were also seen in the dorsal cortical visual pathway, namely, the inferior parietal lobule and the prearcuate region of the frontal lobes. Subcortically, both the body and the posterior portion of the head of the caudate nucleus, known to receive input from posterior parietal and prearcuate frontal cortex, showed right hemispheric reductions in LCGU. Performance on the discrimination task led to an asymmetrical increase in LCGU in structures associated with the active hand and to a symmetrical increase in structures associated with the act of drinking. In visual areas, each

condition of visual stimulation produced essentially the same result. In certain subcortical visual areas, however, the monkeys performing the visual discrimination task showed a lack of left-right hemispheric asymmetries compared to those of the passively stimulated group. Thus, in the body and head of the caudate nucleus, the lateral and basal amygdala, and the medial pulvinar, left-right asymmetries virtually disappeared in the actively discriminating animals, due mainly to increases in right hemispheric activity presumably related to asymmetrical somatosensory input from the active hand. Since visual activation of these regions in the left hemisphere was balanced by somatosensory activation of the same regions in the right hemisphere, it appears that these are zones of sensory convergence serving multimodal functions.

One of the most striking results of this experiment was that the limits of cortical visual tissue were marked by sharp changes in LCGU, and these limits were highly consistent among the animals. Computer-enhanced images of the autoradiographic brain sections were examined and the exact borders of visually related tissue in the parietal and temporal lobes were delineated. We found that the cortical visual-nonvisual borders outlined more visual tissue than expected in both the parietal and temporal lobes.

Behind the junction of the lunate and the intraparietal sulcus all cortical tissue is related to vision. In front of this junction, nonvisual tissue first appears in the superior parietal lobule. This tissue is probably related to somatic sensation. Where the lateral fissure begins, auditory tissue in this fissure and on the superior temporal gyrus separates the visual tissue of the two cortical visual pathways: the dorsal pathway traversing the posterior parietal lobe and the temporal pathway traversing the inferior temporal lobe.

In the parietal lobe, the upper border is always within the intraparietal sulcus, about halfway down the upper bank caudally and closer to the fundus rostrally. The lower border moves out of the lateral fissure and remains on the cortical surface close to the upper lip of the lateral fissure, and then it moves into the intraparietal sulcus rostrally. The rostral limit of visual tissue is within the intraparietal sulcus, about 5mm behind its anterior tip.

In the temporal lobe the upper border is always within the superior temporal sulcus, generally about halfway down the dorsal bank caudally but within the fundus rostrally. The lower border moves from the calcarine fissure to the hippocampal sulcus (where it continues midway along its length) and then turns laterally to enter the occipitotemporal sulcus and finally the fundus of the rhinal sulcus.

These visual-nonvisual borders generally appear at zones of cytoarchitectonic transition described by Bonin and Bailey. For example, in the parietal lobe, a visual-nonvisual border appears on the lateral surface near the zone of transition between areas PG and PF and on the medial surface between prestriate area OA and parietal area PE. Also, in the temporal lobe, visual-nonvisual borders appear in the transition zones between TF and TH, TE and TH, and TE and TG. Finally, inside the expanse of visually related

cortex, metabolic borders appeared to separate architecturally different subareas, as in the lower bank of the intraparietal sulcus and in the upper bank of the superior temporal sulcus. These results lend new functional validity to cortical architectonics.

Experiment 2:

Parts of each visual area of the ventral cortical visual pathway are reciprocally connected through the forebrain commissures. Specifically, the representation of the vertical meridian at the OC-OB border as well as selected parts of area OA receive commissural inputs via the splenium of the corpus callosum, while extensive portions of areas TEO and TE receive contralateral input via both the splenium and the anterior commissure. Since the transfer of visual information between the hemispheres is critically dependent on these reciprocal connections, we attempted to localize and to quantify the contribution to vision made by the commissural systems. To do this we prepared monkeys with a unilateral optic tract section alone leaving the forebrain commissures intact. The 2-deoxyglucose method was applied one month postoperatively under the two behavioral conditions described in experiment 1. The commissural contributions to vision were inferred from differences in LCGU between the deprived hemispheres of these monkeys and those from experiment 1 with the forebrain commissures sectioned.

An extensive computerized quantification of the ventral cortical visual pathway of the animals from experiment 1 and 2 revealed that in the intact hemisphere all animals showed a sequential decline in LCGU along the cortical visual pathway from a high of 66 $\mu\text{moles}/100\text{g}/\text{min}$ in area OC to a low of 47 in anterior TE. Moreover, there were no statistically significant differences in these measures resulting either from the differences in conditions of visual stimulation or in surgical preparation. In the deprived hemisphere there were again no significant differences due to the differing behavioral conditions. There was, however, a statistically significant interaction between surgical preparation and cortical area. In areas OC through TEO, LCGU averaged 50% of that in the intact hemisphere for all animals. In area TE, however, LCGU remained at 60% of that in the intact hemisphere in animals with sectioned commissures but increased to an average of 85% in animals with the commissures intact. This increase in LCGU clearly reflects the functional contribution of the forebrain commissures to vision, but, surprisingly, only to area TE and not to the posterior zones of the pathway, areas TEO, OA, and the OC-OB border. In this prestriate-posterior temporal zone, commissural input may be effective only against a background of direct excitation from the retina. To test this possibility, we prepared a control group of monkeys in which a "blind" right hemisphere was produced by midline section of the optic chiasm combined with occlusion of the right eye, rather than by section of the optic tract. This preparation, however, also failed to reveal increased metabolic levels in the prestriate-posterior temporal zone of the visually occluded hemisphere. Clearly, the presence of background spontaneous neural activity provided by an intact retinal projection did not augment or change the commissural contribution to vision in areas TEO, OA, or the OC-OB border. The likely explanation for the failure of commissural fibers to activate glucose metabolism in the posterior portions of the occipito-temporal pathway has been

suggested by evidence gathered in another project from this laboratory (MH 02036). That evidence strongly suggests that the primary function of the commissures in the posterior portion of the visual system is to provide suppressive rather than excitatory influences on neural activity.

Experiment 3:

We have now begun to use the 2-deoxyglucose method to trace the functional development of the visual system. A series of infant monkeys were prepared with unilateral optic-tract section combined with forebrain commissurotomy at 1 day, 1 week, and 1, 2, 3, and 5 months of age. The 2-deoxyglucose method was then applied during conditions of passive visual stimulation as described in the first experiment. The results show that there are systematic age-related changes both in the absolute level of LCGU within the normal seeing hemisphere and in LCGU differences between the normal left and the deprived right hemispheres.

In all cortical visual areas of the intact hemisphere, LCGU was lowest in the youngest subjects, peaked at 4 months, and then declined in the 6-month-old subject to levels found in adults. As in adults, the intact hemisphere of infants shows a progressive decline in LCGU along the ventral cortical visual pathway from a high in area OC (ranging from 26.1 μ moles/100g/min at 9 days to 88.1 at 4 months) to a low in anterior TE (ranging from 17.6 at 2 days to 59.7 at 4 months). This gradient was present in all subjects, but was shallowest in the two youngest.

The deprived hemisphere showed reduced LCGU relative to the normal hemisphere in all areas of the cortical visual pathway at all ages. Also at all ages, hemispheric differences were greatest in area OC and smallest in anterior TE. These differences, however, varied systematically with the age of the animal. Thus, for each cortical area, the relative difference between the normal and deprived hemispheres was smallest in the youngest subjects and approached the differences seen in adults only at about four months of age, the time at which LCGU appeared to peak.

This finding that adult levels of metabolic activity are not reached until about 4 months of age is consistent with behavioral data (see Project MH 02038) indicating that the neural capacity for visual object recognition is probably not developed until about this time.

SIGNIFICANCE TO MENTAL HEALTH RESEARCH:

The goal of this research is the understanding of normal function within the central nervous system, a goal that will in time aid in diagnosing and treating the abnormal function underlying a variety of mental disorders. Since it has been widely demonstrated that metabolic activity, in the form of glucose utilization, and functional activity are highly correlated within the central nervous system, the 2-deoxyglucose method provides a unique method of relating neural structure and function. This method permits for the first time both the visualization and quantification of local levels of metabolic activity simultaneously throughout the entire brain in animals studied either

under normal conditions or following experimental intervention. The results continue to provide important insights into the role of various cerebral structures, both cortical and subcortical, in particular behaviors. Our initial studies have contributed information regarding both primary and higher-order visual processing, the understanding of which is critical for the diagnosis and treatment of sensory, perceptual, and mnemonic disorders related to vision.

PROPOSED COURSE OF RESEARCH:

Our immediate goals are to continue the investigation of the extended visual system in primates including 1) delineation of the visual-nonvisual borders in the frontal lobes and 2) a more extensive quantification of the dorsal visual pathway. Moreover, we will expand the investigation to include additional conditions of visual stimulation. Specifically, we plan to measure metabolic activity within the visual system while visual memory is being taxed. To do this, the 2-deoxyglucose method will be applied while monkeys are performing an object recognition task designed to tax the memory system as well as the visual system throughout the experimental session. In this way we hope to see increased LCGU in parts of the limbo-thalamic system that were not seen in our purely visual studies.

We also plan to continue our investigation of the development of the visual system in infant monkeys, first completing the normative study under conditions of passive visual stimulation and then attempting to parcel out developmental differences between the "habit" and the "memory" systems (see Project MH 00478). Ultimately, our goal is to apply the 2-deoxyglucose method to the study of a variety of behavioral processes in the adult and infant monkey, including perception, attention, memory, emotion, and volition for the purpose of identifying the various structures involved in these different behaviors and quantifying the degree of their participation.

(The principal investigator of this on-going research project assumed part-time guest worker status in May 1983; consequently, progress has been slowed due to limited man-hours.)

PUBLICATIONS:

Macko, K.A. and Mishkin, M. Metabolic mapping of higher visual processing in primates. J. of Assoc. Res. Nerv. and Mental Disease (in press), 1984.

Mishkin, M., Ungerleider, L.G., and Macko, K.A. Object vision and spatial vision: Two cortical pathways. Trends in Neuroscience 6: 414-417, 1983.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02035-04 LN

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Anatomy of the primate visual system

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

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COOPERATING UNITS (if any)

None

LAB/BRANCH

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SECTION

INSTITUTE AND LOCATION

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TOTAL MAN-YEARS:

4.0

PROFESSIONAL:

1.0

OTHER:

3.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

To trace the flow of visual information out of the occipital lobe, we have undertaken a series of studies using neuroanatomical tracing techniques combined with electrophysiological recording. Our goal is to identify the multiple visual areas within the prestriate cortex of the macaque, explore their organization, and map their projections forward into both the temporal and parietal lobes. In these studies, we have also utilized a new myeloarchitectural stain that, for the first time, clearly distinguishes among the multiple prestriate areas. Our results indicate that striate cortex is the major source of the two cortical visual systems. The first system begins with the striate projections to the second and third visual areas, V2 and V3. Both V2 and V3 project in turn to V4. These three prestriate areas are arranged in adjacent belts that nearly surround the striate cortex, and, like the striate cortex, each belt contains a topographic map of the visual field. Area V4 projects in turn to the inferior temporal cortex. The second system begins with both striate and V2 projections to area MT, which is also topographically organized. However, in contrast to V4, which provides a major link forward from striate cortex into the temporal lobe, our results on MT indicate that it provides a major link forward from striate cortex into the parietal lobe via its projections to four additional areas in the superior temporal and intraparietal sulci. Thus, one system of projections out of striate cortex is directed ventrally into the temporal lobe, while a second is directed dorsally into the parietal lobe. Based on correlations with out neurobehavioral studies, we propose that these two systems mediate object vision and spatial vision, respectively.

PROJECT DESCRIPTION:

Converging evidence from our earlier neurobehavioral, physiological, and anatomical studies indicates that the striate cortex in the monkey is the source of two corticocortical, multisynaptic pathways. One of these follows the course of the inferior longitudinal fasciculus, interconnects the striate, prestriate, and inferior temporal areas, and appears to be important for object vision. The other follows the course of the superior longitudinal fasciculus, interconnects the striate, prestriate, and inferior parietal areas, and is critical instead for spatial vision. Although visual information must reach the inferior temporal and inferior parietal cortex to enable their participation in object vision and spatial vision, respectively, the complex circuitry through which this information is transmitted has yet to be unraveled. We have undertaken to examine the details of the connections within these two cortical visual pathways, beginning with an analysis of the projections of the striate cortex itself.

Experiment 1: Projections of Striate Cortex

To determine the full extent of prestriate cortex that receives striate projections, we prepared one series of monkeys (Macaca mulatta) with large lesions that covered, collectively, all of striate cortex and then processed the brains for anterograde terminal degeneration. To determine the topographic organization of the striate projection fields within prestriate cortex, we prepared a second series of monkeys with tritiated amino-acid injections into selected sites throughout striate cortex, including the representation of the center of gaze and eccentricities ranging from 0.5° to greater than 60° in both the upper and lower visual fields.

The results indicate that striate cortex (cytoarchitectonic area OC), or V1, projects topographically to two major fields within prestriate cortex: V2, a circumstriate cortical belt, and MT, located on the posterior bank and floor of the superior temporal sulcus. In addition, the part of striate cortex representing the lower visual field projects to a part of V3, a field anterior and adjacent to V2. There is also a sparse projection from striate cortex to V3A, located at the fundus of the posterior intraparietal sulcus. V2 corresponds to cytoarchitectonic area OB, while MT, V3, and V3A are all located within area OA. This experiment is now complete and the results are being prepared for publication.

Experiment 2: Projections of Area V2

Our approach to studying the projections of V2 was the same as that used in striate cortex and again employed both degeneration and autoradiographic tracing techniques. First, we determined the full extent of the projection fields by making large lesions that covered, collectively, all of V2. Second, the topographic organization of the fields was determined by injecting tritiated amino acids into selected V2 sites. In these experiments, all injection sites were identified electrophysiologically. Thus, by recording the activity of neurons from the microsyringe needle and mapping receptive fields, we were able to make our injections into portions of V2 that represent

known parts of the visual field. The 14 sites we injected included the center of gaze and positions ranging from about 2.5° to greater than 60° in both the upper and lower visual fields.

Our results indicate that V2 projects to two visual areas located anterior to it, V3 and V4. Together, these three prestriate areas are arranged in adjacent belts that nearly surround the striate cortex, and, like striate cortex, each belt contains a full representation of the contralateral visual field, with the upper visual field located ventrally in the hemisphere, the lower visual field dorsally, the central visual field laterally, and the peripheral visual field medially. The vertical and horizontal meridians of the visual field are represented alternately at the anterior borders of V1, V2, and V3, respectively.

In addition to sending projections to V3 and V4, V2 sends a projection to MT which is in topographic register with the one that MT receives directly from striate cortex. There is also a projection from V2 back to striate cortex. In contrast to the forward projections of V2 to areas V3, V4, and MT, which terminate predominantly in layer IV and the deep part of layer III and often appear as columns, the backward projection of V2 to striate cortex terminates in layer I, II, and V. The projections of the striate cortex itself to areas V2, V3, V3A, and MT are all characterized by the laminar pattern of forward projections.

Experiment 3: Projections of V4

We have recently begun to explore the projections of V4. In these studies, we have placed multiple tracers, both anterograde and retrograde, into V4 of the same animal, thereby enabling us to compare the projections from the different visual field representations of V4 within a single brain. Our preliminary results indicate that V4 has widespread projections to both areas TE0 and TE in the inferior temporal cortex. Whereas TE0 receives projections from only the central visual field representation of V4, TE appears to receive a very complex pattern of projections from V4, with a single site in V4 projecting to several separate fields which interdigitate with projection fields of other sites in V4. Thus, there may be a mosaic of visual areas within the temporal lobe, a previously unsuspected possibility, and one that we are currently investigating. As described below, our results on the projections of MT suggest that the the parietal lobe also may contain a mosaic of visual areas.

Experiment 4: Projections of MT

To determine the projection fields of MT, we restricted ourselves to autoradiographic tracing methods, inasmuch as MT is contained within the superior temporal sulcus and is impossible to remove surgically without inadvertent damage to the surrounding cortex. Our tritiated amino acid injections covered, collectively, most of MT and included the center of gaze and positions ranging from 8° to 25° in both the upper and lower visual fields. As in V2, the injection sites in MT were identified electrophysiologically.

Our results indicate that MT provides a major link from striate cortex into the parietal lobe via its projections to four separate areas located in the intraparietal and superior temporal sulci. Each of these projection zones, like MT itself, has a distinctive myeloarchitecture, which we have used to define the borders of these areas. Within the intraparietal sulcus, a posterior projection zone begins in the annectent gyrus caudally and extends along the fundus of the posterior third of the intraparietal sulcus. We had found earlier that this zone also receives a sparse projection from striate cortex. The other projection zone in the intraparietal sulcus lies in the anterior two-thirds of the sulcus, extending from the fundus onto the posterior bank; this zone is located in cytoarchitectonic area PG. Within the superior temporal sulcus, one projection zone of MT is located on the anterior bank of the sulcus, bordering MT medially, and the other is located in the sulcal floor, bordering MT anteriorly. There is considerable overlap in the projections to these medial and anterior zones from all parts of MT, indicating a convergence of inputs representing widely separated parts of the visual field.

The finding of multiple projection zones of MT in both the superior temporal and intraparietal sulci raises the question of what role these areas play in visual function. To explore this question, we have begun recording the electrophysiological properties of neurons within MT's projection zones and comparing these properties with those of neurons in MT itself. Our preliminary results indicate that, like MT, the projection zone medial to MT in the superior temporal sulcus is characterized by a high proportion of directionally selective neurons. Thus, the analysis of direction-of-motion information in MT appears to be elaborated further in this particular projection zone. By contrast, few neurons in the projection zone anterior to MT in the superior temporal sulcus are directionally selective. Interestingly, the receptive fields of neurons in both zones are larger in size than those of MT neurons, a finding consistent with the convergent input that these two areas receive from all parts of MT. For the area medial to MT, such convergence is presumably related to the large size of the receptive field over which information about direction of motion is analyzed. For the area anterior to MT, the neuronal properties are still unknown.

Experiment 5: Subcortical Projections of Visual Cortex

Anatomical material from Experiments 2 and 3 was used to investigate the location and topographic organization of the subcortical projections from areas V2 and MT and to compare them with those of the striate cortex. Our results indicate that both V2 and MT, like striate cortex, project topographically to the inferior and lateral pulvinar, the superior colliculus, and the reticular nucleus of the thalamus. MT projects, in addition, to the putamen, caudate, claustrum, and pontine grey. Interestingly, in our ongoing experiments we have found that V4 also projects to these striatal and pontine structures. Thus, the subcortical projections of MT and V4 are more extensive than those of either striate cortex or V2. The considerable overlap in the subcortical projections from MT and V4 suggests that the contribution of each area to subcortical processing lies not in a unique set of subcortical projections but rather in the unique information each supplies, namely,

direction of stimulus motion for MT and stimulus form and color for V4 (see Project MH 02036). In addition, the fact that the projections from V4 to the putamen, caudate, and claustrum are topographically organized indicates that each of these structures contains a map of the visual field.

SIGNIFICANCE TO MENTAL HEALTH RESEARCH:

An understanding of the basic mechanisms mediating normal visual perception and memory is the first step in the diagnosis, alleviation, and prevention of sensory, perceptual, and mnemonic disorders. To this end, we have been exploring the projections of striate cortex both to prestriate "association areas" and to subcortical structures. Our goal is to unravel the complex system of projections to the still higher-order visual areas located within the parietal and temporal cortex, areas critical for spatial vision and object vision, respectively. The combined use of axonal transport techniques and electrophysiological recording provides a powerful tool for tracing neural connections within these central visual pathways. In addition, the recent development of highly selective histological stains may give us the opportunity for the first time of identifying the higher-order visual areas in the human brain that we have identified in the monkey.

PROPOSED COURSE OF RESEARCH:

To understand the role of visual association cortex in perception and memory we must identify the multiple functional areas that comprise this cortex, delineate their topographic organization, and explore the complex circuitry of their interconnections. So far, we have discovered that striate cortex is the source of two divergent cortical pathways, each with its own set of hierarchically organized prestriate association areas. We have found that the projections of both pathways can be traced stepwise to the still higher-order visual areas located within the temporal and parietal lobes. Our most recent studies suggest that both the temporal and parietal lobes may each consist of multiple visual areas, and we intend to investigate this possibility intensively in the coming year. A major question for the future will be how the object and spatial information carried in these two separate pathways are subsequently integrated anatomically to yield a unified percept. Ultimately, we will explore the links of both pathways to affective, mnemonic, cognitive, and motor systems by examining the projections of the multiple visual association areas to the limbic system, the prefrontal cortex, and the striatum.

PUBLICATIONS:

Ungerleider, L. G. Contrasts between the corticocortical pathways for pattern and spatial vision. In C. Chagas (Ed.) Study Group on: Pattern Recognition Mechanisms, The Pontifical Academy of Sciences, Vatican City (in press), 1984.

Ungerleider, L.G., Desimone, R., Galkin, T.W., and Mishkin, M. Subcortical projections of area MT in the macaque. J. Comp. Neurol. 223: 368-386, 1984.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02036-04 LN

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neural representation of visual stimuli in extrastriate cortex

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

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TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

2.5

1.0

1.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The neural mechanisms for the visual recognition of objects extend beyond striate cortex into multiple extrastriate cortical areas within the occipital, temporal, and parietal lobes. To understand the neural mechanisms of perception and memory in these areas, we are studying 1) passive sensory coding by single neurons in the immobilized monkey 2) dynamic aspects of coding by neurons in an awake monkey performing a visual discrimination and memory task and 3) the functional architecture of the cortex utilizing local metabolic mapping techniques. In one area, area V4, we have found that neurons code many local features of objects, such as the length and width of contours, textures, and colors. As neurons in this area are sensitive to form and color differences between a stimulus and its background, they may play a role in separating figure from ground. One of the primary functions of the corpus callosum in area V4 appears to be to integrate the figure/ground mechanism across both halves of the visual field. Unlike neurons in parietal cortex, neurons in V4 are affected very little by the spatial location of the animal's attention. In inferior temporal cortex we found that over half the neurons were tuned to a set of shape descriptors that can be used to code object shape. Since different neurons are tuned to different descriptors, a population of inferior temporal neurons could code any shape. Finally, we have found that another extrastriate area, area MT, is specialized for analyzing stimulus motion and contains direction-of-motion columns similar to the orientation columns discovered in primary visual cortex. Whereas area V4 and inferior temporal cortex form part of an occipito-temporal system for object recognition, area MT contributes to an occipito-parietal system for spatial perception.

PROJECT DESCRIPTION:

Previous work in this and other laboratories has shown that the cortical systems involved in perception and memory include multiple visual areas that lie beyond the striate cortex within the occipital, parietal, and temporal lobes. To begin to understand the neural mechanisms of perception and memory in these extrastriate areas, we have taken three interrelated approaches. The first is to establish the basic sensory information coded by neurons in the different extrastriate areas. To study the passive visual properties of neurons in these areas, unaffected by eye movements or the changing state of the animal, we are recording neural activity in the immobilized, lightly anesthetized macaque. A second, more recent, approach has been to study the dynamic aspects of stimulus coding by neurons in an awake monkey performing a visual discrimination and memory task. Finally, the most recent approach has been to explore the functional architecture of the visual association cortex utilizing local metabolic mapping techniques.

Passive Sensory Coding

Experiment 1: Neural mechanisms for form and color

Anatomical experiments in our laboratory have shown that visual area V4 is a central station in the pathway from the primary visual cortex to the object recognition system of the temporal cortex. We have recorded from V4 neurons in the immobilized, anesthetized macaque. Although V4 was originally thought to be exclusively concerned with color, we found that V4 neurons are as sensitive to stimulus form as they are to color. In fact, V4 neurons are at least as sensitive as neurons in the primary visual cortex to such features of form as the orientation and size of contours and the spatial frequency of sine wave gratings. The properties of many V4 neurons suggest they may also play a role in texture discrimination. Ours are the first findings which indicate that V4 is far more than a "color processing area".

What are the special contributions of V4 to perception? We have found that unlike neurons in the primary visual cortex, the receptive fields of neurons in V4 are surrounded by large, silent suppressive regions with specific form and color properties. Stimuli placed outside of the receptive field do not elicit any response from a V4 neuron, yet these stimuli are able to completely suppress the response of the neuron to a similar receptive-field stimulus. Thus, many V4 neurons respond to a stimulus only if it stands out from its background on the basis of a difference in color or form. These neurons may thus play a role in separating 'figure' from 'ground', a fundamental task in visual perception.

The results from our neural recording experiments, in conjunction with anatomical findings from our laboratory, have led us to reject the "division-of-labor" theory of object recognition. According to this theory, the primary visual cortex sends parallel information about an object on the retina to a large number of association areas in each of which processes a different feature of the object, such as color in V4 and stereopsis in V2. By contrast, anatomical experiments in our laboratory indicate that the cortical

areas of the object recognition pathway are primarily organized in a serial hierarchy. Area V4 plays a central role in this hierarchy, and our recording experiments show that all aspects of a stimulus are analyzed in V4, just as they are in the primary visual cortex. Thus, we propose that parallel processing of object features occurs within each area rather than across areas. This new model focuses attention on the functional neural architecture within each area that mediates this parallel processing. In experiments which are described later in this report, we have begun to examine the functional architecture of the different extrastriate areas.

Experiment 2: The role of the corpus callosum.

Like other prestriate areas, V4 contains a representation of the contralateral visual field. Within the central visual field, V4 receptive fields rarely extend more than 10° across the vertical meridian into the ipsilateral visual field. Yet, V4 receives heavy commissural projections from the opposite hemisphere not limited to the representation of the vertical meridian. What is the purpose of these projections? As described above, we have found that receptive fields of V4 neurons have large suppressive surrounds. To test whether the commissural projections might contribute to the suppressive surrounds, we measured the extent of the suppressive surrounds of individual V4 neurons within the ipsilateral hemifield. We found that even though the excitatory receptive fields of V4 neurons were confined to the contralateral hemifield, the suppressive surrounds extended up to 160° across the vertical meridian into the ipsilateral visual field. The ipsilateral suppression was eliminated following section of the corpus callosum. Thus, the commissural inputs to V4 (and presumably other prestriate visual areas) appear to be largely suppressive and may serve to integrate the figure/ground mechanisms in the two hemispheres. Now that we have established this new phenomenon, we plan to investigate the pharmacological and synaptic mechanisms that underlie the suppressive action of the corpus callosum as well as the perceptual function that it mediates.

Experiment 3: Neural mechanisms for shape analysis

Anatomical experiments in our laboratory have shown that inferior temporal (IT) cortex is the last exclusively visual area in the cortical object recognition system, and our neurobehavioral experiments have shown that IT cortex plays an especially important role in the visual memory of objects.

In our first study of IT neurons, we surveyed their response to a large variety of stimuli, both simple and complex. We found that, like neurons in other visual areas such as V4, most IT neurons give at least a small response to many stimuli but respond better to some stimuli than to others. Presumably, therefore, the neural representation of objects in IT cortex is reflected in the pattern of activity across a population of cells and not in the activity of individual cells that respond only to specific objects.

In our survey of IT neuronal responses we found that many cells seemed more sensitive to the overall shape of stimuli than to the location and quality of individual edges and contours. Therefore, in a subsequent study we examined

how IT cortex might extract information about the overall shape of an object from information about its boundary. In order to represent shapes in terms of local boundary orientation, we adopted a method that is used in computer pattern-recognition systems. The method depends on extracting a set of periodic features, known as the Fourier Descriptors, from the boundary of the object. Any shape is fully described by its set of Fourier Descriptors, or FDs, and a smaller set of only the low-frequency terms can often provide the 'gestalt' of a shape. Thus, the FDs are a powerful and efficient alphabet for representing and classifying shapes.

To explore the shape selectivity of IT neurons, we created a set of stimuli from single FDs. If IT neurons function as 'bandpass filters' for shape, one would expect different IT neurons to be tuned to different FD stimuli, and the tuning should be relatively independent of the size and position of the shape on the retina. The activity of a set of such neurons could specify or code any complex shape.

About half of the IT neurons we studied were tuned to different FD stimuli. For two-thirds of the tuned cells, the shape of the tuning curve remained invariant over changes in the size of the stimulus and in its position on the retina. These results support the possibility that the visual system, and inferior temporal cortex in particular, use periodic shape descriptors in classifying objects. Both our survey of IT neuronal responses and our investigation of shape selectivity are complete. Since many of the leads from our findings are being actively pursued by other laboratories, we will devote less effort in the future to stimulus coding in IT cortex and more to other lines of investigation.

Experiment 4: Mechanisms for spatial perception

Area MT in the macaque appears to play a smaller role than areas V4 and IT in object recognition but may play a greater role than they do in spatial perception. Anatomical experiments in our laboratory have shown that MT is a central station in the pathway from the primary visual cortex to the spatial perceptual system of the parietal lobe. Although it had been shown by other laboratories that MT neurons are sensitive to the direction of stimulus motion, this is not an exclusive property of MT. To gain insight into the special contribution of MT to motion analysis, we explored the columnar organization and visuotopic organization of MT.

We discovered that area MT contains a columnar architecture for analyzing the direction of stimulus motion. The representation of direction of motion in MT is strikingly similar to the representation of orientation in the primary visual, or striate, cortex. Even the size of the columnar systems is similar: 180 degrees of direction of motion in MT is represented within a piece of cortex 400 to 500 microns wide, the same size as the representation of 180 degrees of stimulus orientation in striate cortex. These results suggest that just as the analysis of stimulus orientation is a fundamental function of striate cortex, the analysis of stimulus direction of motion is a fundamental function of area MT. Ours is the first physiological demonstration of a columnar system outside that of the primary visual cortex. If we could find

such an organization for other stimulus dimensions within the multiple visual association areas, this would provide us with the best evidence yet of the functions of these areas. Our study in area MT is now complete and published. In subsequent studies we plan to continue to explore the functional architecture of MT and other extrastriate visual areas using metabolic mapping techniques.

In a separate study, we examined the fine-grained visuotopic organization of MT. We found that while MT is about ten times smaller than the primary visual cortex, MT receptive fields are about ten times larger. Based on receptive field size, magnification factor and scatter in MT, we were able to calculate the point-image area, which is the area of cortex activated by a single point in the visual field. Surprisingly, although MT is much smaller than striate cortex the point-image area is the same. Since the size of columns is the same in MT and striate cortex, our results indicate that a visual stimulus is analyzed by the same number of columns in both MT and striate cortex. The number of columns activated by a stimulus may be a fundamental principle of organization in the visual cortex. This idea will be tested in other visual areas.

Dynamics of sensory coding

At any given moment, our retinas contain the images of many different objects and we must decide which object or objects are relevant to our goals and behavior. 'Selective attention' is usually the name given to the mechanism that selects which object enters consciousness, is remembered, or guides our behavior. How soon such selection occurs in visual processing is a matter of current debate. Some argue that attention occurs very early and plays an important role in the initial processing of stimuli, whereas others argue that attention occurs very late, at the point of memory storage or initiation of behavior after all stimuli have been decoded.

We have developed a paradigm to investigate the effects of attention on neurons in the visual association cortex. In our paradigm, the monkey first fixates a spot of light. A sample stimulus and an irrelevant stimulus are then presented simultaneously. Either the sample or the irrelevant stimulus is within the receptive field of the neuron studied and the other is outside the field. A short time after the sample and irrelevant stimuli are removed, a 'match' stimulus is presented at the same site as the sample, and the monkey is required to signal whether the match is the same or different from the sample. Thus, by placing either the sample stimulus or the irrelevant stimulus within the receptive field, we can control whether the monkey is attending or not attending to the stimulus to which the neuron responds. By using variations of this paradigm we can also investigate whether the use of a stimulus as the cue for a motor response affects the neuronal response and whether the mnemonic aspects of the task are related to the neuronal response.

Our results so far are preliminary, but it appears that selective attention occurs very late in visual processing. We are currently trying to determine in which of the visual areas selective attention plays a role.

Local Metabolic Mapping

The results from our recording experiments suggest that object recognition depends not so much on the activity of individual neurons as on the distribution of activity across a large population of neurons. Metabolic mapping with the 2-deoxyglucose technique, developed by Sokoloff in the Laboratory of Cerebral Metabolism, is a powerful tool for investigating the distribution of activity over wide areas. We are using a modification of the Sokoloff technique, developed by S.J. Schein in the NEI, which has a resolution that is nearly at the single neuron level. Over the past year, we have established procedures for using the Schein method in awake macaques and marmoset monkeys viewing a computer-generated pictorial display. The marmoset is a particularly useful animal for this procedure since it is nearly lissencephalic and its cortex can be flat-mounted. Thus, we can view the distribution of activity throughout its entire cerebral cortex in a single section. Over the next year, we will begin to study the effects of sensory coding, selective attention, and visual memory on the distribution of cortical activity.

SIGNIFICANCE TO BIOMEDICAL AND BEHAVIORAL RESEARCH:

The primate, including man, is a highly visual animal. Thus, it is not surprising that perhaps half of the primate cerebral cortex is devoted directly or indirectly to visual processing. Consequently, the study of the neural mechanisms of vision is not only of fundamental importance for our understanding of visual perception and memory but also for our understanding of cerebral function in general. The extrastriate visual areas described in this project are of particular importance to the field of neurobehavioral research because they contain the neural mechanisms for visual perception and memory, and they are the direct source of nearly all the visual information to the affective and motivational mechanisms of the limbic system.

PROPOSED COURSE OF RESEARCH:

Our findings in the past year that neurons in one prestriate area are organized within direction-of-motion columns, that neurons in a different prestriate area code contour and color, and that neurons in inferior temporal cortex appear to code object shape all indicate that the study of single neurons can provide valuable insight into the neural mechanisms of perception and memory. Clearly we have only scratched the surface. We hope to follow the flow of information about motion into the parietal visuospatial system and study how parietal neurons use that information to code the spatial relations among objects. In addition, within the system for pattern vision, we plan to study how prestriate neurons use information about contours and colors to separate figure from ground. Within both the visuospatial and object recognition systems, we will study the roles that attention and memory play in neural processing. Finally, utilizing newly refined metabolic mapping techniques, we will study the functional architecture of the extrastriate cortex.

PUBLICATIONS:

Albright, T.D., Desimone, R., and Gross, C.G. Columnar organization of directionally selective cells in visual area MT of the macaque. J. Neurophysiol. 51: 16-31, 1984.

Desimone, R., Albright, T.D., Gross, C.G., and Bruce, C. Stimulus selective properties of inferior temporal neurons in the macaque. J. Neurosci. (in press), 1984.

Desimone, R., Schein, S.J., and Albright, T.D. Neural mechanisms for form, color and motion analysis in prestriate cortex of the macaque. In C. Chagas (ed.): Working Group on Pattern Recognition Mechanism, Pontifical Academy of Sciences, Vatican City (in press), 1984.

Desimone, R., Schein, S.J., Moran, J., and Ungerleider, L. Contour, color and shape analysis beyond the striate cortex. Vis. Res. (in press), 1984.

Gross, C.G., Desimone, R., Albright, T.D., and Schwartz, E.L. Inferior temporal cortex as a visual integration area. IBRO Monograph Series (in press), 1984.

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Schwartz, E.L., Desimone, R., Albright, T.D., and Gross, C.G. Shape recognition and inferior temporal neurons. Proc. Nat. Acad. Sci. 80: 5776-5778, 1983.

NOTICE OF INTRAMURAL RESEARCH PROJECT

201 MH 02037-03 LN

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Functional anatomy of the somatosensory cortex of the monkey

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

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1.5

PROFESSIONAL:

1.0

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A pathway by which somatosensory information could reach the limbic structures in the temporal lobe known to be critical for tactile memory has now been delineated. To trace this pathway, the anatomical connections of electrophysiologically identified somatosensory fields lying in or near the lateral sulcus of the macaque monkey were investigated with both anterograde and retrograde axonal transport techniques. The data show that a series of parallel tactile processing pathways converge on the insular cortex; this region, in turn, projects directly to the amygdala and indirectly to the hippocampus via the rhinal cortex, thus linking the somatosensory cortices with the limbic structures of the temporal lobe.

The functional relationships among the various somatic subfields are still unknown. In particular, the response properties of the neurons of the insula have not been studied in any detail and the role of corticocortical projections arising in the primary somatosensory cortex (SI) is also unclear. Electrophysiological studies of these problems now in progress suggest that the posterior insula has neuronal properties characteristic of a higher-order somatic processing area. Further, the somatic fields in the lateral sulcus appear to be dependent on SI for their somatic input because neurons in these fields no longer respond to tactile stimulation following ablation of SI.

PROJECT DESCRIPTION:

Work in this laboratory has shown that the amygdala and hippocampus are critical not only for visual memory but also for tactual memory. Though these studies suggested that the second somatosensory area (SII) and the insular cortex may act as relays for the transfer of somatic inputs from the first somatosensory area (SI) to the limbic system, there are a number of other somatosensory fields in or near the lateral sulcus that could serve this relay function. Because little is known about the connectivity or other properties of these fields, we have undertaken such study with the goal of delineating (I) the route via which somatosensory information reaches the limbic structures critical for memory, (II) the corticocortical interrelations of the somatic fields involved, (III) the thalamic relationships of these fields, (IV) their single-unit response properties, and (V) the sources of somatic input to the higher-order somatic processing areas. Further (VI), similar studies have been undertaken in the cat, in collaboration with R.S. Waters at Rockefeller University, in order to obtain data from a second species and also to examine the motor projections of somatic cortical fields.

Methods employed:

Single-unit and multi-unit recording techniques were used to identify the specific cortical fields in the lateral sulcus of the macaque that are activated by somatic input. These fields include the second somatosensory area (SII), area 7b, the retroinsular area, and the granular and dysgranular insular fields. In early experiments, after a particular field was mapped, an injection of either tritiated amino acids (a mixture of proline and leucine) or HRP was made into the hand or digital representation within it to trace its connections. In later experiments, injections into the representations of other body parts have been made to help outline the projection zone of each field. Accurate placement of the injection was ensured by either i) injecting through the recording pipette by iontophoresis or ii) recording from a microelectrode cemented to the needle of the injection syringe.

Histological identification of cortical fields has been improved through processing of adjacent sections to reveal either cell bodies, with a standard Nissl stain, or axons, with a sensitive silver stain we have developed for bulk use.

Preliminary physiological studies of lateral sulcus neurons have been performed in immobilized monkeys, which were lightly anesthetized, and more complete studies are being performed in awake monkeys seated in a primate chair.

In another group of monkeys the cortical fields comprising the first somatosensory area have been ablated in one hemisphere. In some of these animals the corpus callosum and anterior commissures were split and in others the commissures were left intact. Single-unit recording studies of SII and the granular insular field ipsilateral to the lesion were performed to determine if tactile stimulation of the body surface could drive units in these two fields in the absence of SI cortex.

Major findings:

I. Corticocortical Connectivity:

Using the combined recording-injection techniques described above, we have placed injections into SI, SII, area 7b, area 5, the retroinsular field (Ri), and the granular (Ig), dysgranular (Id), and agranular (Ia) insular fields. By combining the data concerning anterograde projections derived from the tritiated amino-acid injections and retrograde projections derived from the HRP injections, we have demonstrated reciprocal connections between: SII and Ri, SII and area 7b, SII and Ig and Id, and Ri and Ig. Also, we have confirmed previously reported reciprocal projections between SI and SII and demonstrated reciprocal projections between area 5 and both Ri and area 7b. Finally, anterograde labeling resulting from HRP injections into the insular fields has confirmed recently reported projections from Ig, Id, and Ia to the amygdala and from Id and Ia to the prorhinal and perirhinal cortical areas. These areas, in turn, are known to send projections to the hippocampus.

Our studies thus demonstrate that tactual information may reach the amygdala and hippocampus via relays in the granular and dysgranular insular fields, which receive their somatic cortical inputs from SII and Ri. A ventrally directed cortico-limbic pathway originating in SI may therefore be important for the perception and memory of somatosensory stimuli. This possibility will be examined directly in a series of neurobehavioral studies.

II. Laminar Patterns of Termination:

Three different laminar patterns of termination of the corticocortical projections described above were seen. Each pattern depended on the field into which the injection was made and the field to which the injected field projected. Though similar patterns have been described in other areas of the cortex, only one has previously been reported in the somatosensory system.

This pattern consists of a heavy band of labeled terminals in layer IV, with progressively lighter labeling, indicative of fewer terminals, in the supragranular layers, III, II, and I. There is a light band of terminal labeling in layer VI paralleling that seen in layer IV. This pattern has been described for the projections from SI to SII and area 5, and from area 5 to Ri and 7b. It is similar to the forward projections (i.e. outward from striate cortex) seen in the visual system.

The second pattern is analogous to the one described in the visual system as a backward projection (i.e. towards the striate cortex). Its most striking characteristics are a complete absence of labeling in layer IV and heavy labeling in layer I. Additional labeling is seen in layer VI and sometimes in layers III and II. The projections from SII to SI and Ri, from Ri to area 5, and from Ig to SII and Ri are all of this type.

The third pattern, previously described in prefrontal association areas, consists of a single, apparently homogeneous column of labeled terminals extending from layer VI through layer I. Its most striking feature is the

lack of laminar differences in labeling density, in sharp contrast to the so-called forward and backward projections described above. This pattern is seen in the projection from SII to area 7b.

By analyzing the pattern of forward and backward projections, we have been able to determine the probable sequential order in which information is processed in the somatosensory system. The forward direction is SI to SII and area 5, area 5 to Ri and area 7b, area 7b and Ri to SII, and SII and Ri to Ig. SII also projects to Id. Ig and Id then project to the limbic system.

III. Thalamocortical relations:

In conjunction with the above work, the thalamic connectivity of the cortical fields of the somatosensory system was thoroughly examined. This study was required because preliminary findings indicated that the thalamic relations of these cortical fields differ from those described in the literature. By having, for the first time, an appreciation of the full extent of these fields, and by using our combined recording-injection techniques to increase the accuracy of our injections, we have been able to provide a new account of this fundamental anatomical relationship.

There are three major new findings: (1) The second somatosensory area (SII) receives its major thalamic input from the ventroposterior inferior thalamic nucleus (VPI) and additional inputs from the central lateral nucleus and the caudal division of the ventroposterior lateral nucleus (VPLc); the latter inputs, in contrast to previous reports, arise only from the most ventral and caudal portions of VPLc and from the ventroposterior medial nucleus (VPM). The finding in the monkey that SI and SII receive different thalamic inputs is consistent with the hypothesis that they process information in a sequential rather than parallel manner, the latter notion having been based on previous reports that both fields received their thalamic input from one source, VPLc. Because of the importance of determining whether the cortical processing in SI and SII is sequential or parallel we are pursuing this question with additional experiments (see V below). (2) The dysgranular insular field (Id) receives thalamic input from a continuous band of neurons that runs caudally and ventrolaterally from the basal ventromedial nucleus (VMB) through VPI and the posterior nuclei (Po) to the medial edge of the medial geniculate body (MG) and not simply from VPI as previously reported. Additional inputs to Id arise from the intralaminar nuclei and nucleus reuniens and from the medial nucleus of the pulvinar (Pulm). (3) Pulm projects to a number of the cortical somatosensory fields including Id, the granular insula (Ig), and area 7b. Thus, the cortical territory receiving projections from the medial pulvinar includes not only the frontal, parietal, and temporal lobes, as previously reported, but also the insula. The nature and function of this widespread projection are still unknown.

We have confirmed projections to Ig from the supragenulate and limitans nuclei and to the retroinsular area from the posterior group. However, these projections do not conform to previous descriptions because the thalamocortical relay neurons labeled retrogradely by HRP injections into

these fields appear to cross the borders of thalamic nuclei, as in the case of the projection to Id described above.

These findings suggest a revision of the traditional view of thalamocortical organization, which states that a single thalamic relay nucleus projects to a single cortical field. In the somatosensory system, at least, it now appears that each cortical field outside of SI receives an array of inputs from a number of thalamic nuclei and that each thalamic nucleus projects to several cortical fields.

IV. Electrophysiological Studies of Insular Cortical Neurons:

In our exploration of the neuronal properties of the granular insula (Ig) in awake monkeys, we recorded both single and multiple units. Over 500 individual recordings were made through a region extending from the retroinsular area caudally to the level of the arcuate sulcus rostrally. On histological examination, we identified 237 units as having been located in the posterior insula, Ig. Of these Ig units, a minority (32%) could not be driven by any of the stimuli tried in any sensory modality, while the remainder (68%) were driven by innocuous, somatic stimuli. The population of somatic units was composed of two subgroups, one activated by intraoral stimulation (21%) and the other by stimulation of the body surface (79%). Of the latter, 61% were driven by stimuli classified as cutaneous (light touch or stroking) and 39% by stimuli classified as deep (joint movement or pressure on deep structures). There was no convergence between the two submodalities, nor were any of the somatic units responsive to gustatory, auditory, or visual stimuli, though 7 of the body-surface units did respond more intensely when the animal appeared to attend visually to the stimulating object. A prominent characteristic of the body-surface units was bilaterality of their receptive fields (86%).

There were several characteristics which distinguished the anterior from the posterior portions of Ig. Anterior Ig contained most of the units responding to intraoral stimulation (78%) and to stimulation of the face (92%). Anterior Ig also contained 95% of the undriven units, 78% of the units with small receptive fields, and 70% of the units responsive to deep stimuli. The posterior portion of Ig contained all of the whole-body units, i.e. those responding to stimulation of almost the entire body surface. However, more units responding to parts of the body surface exclusive of the head were also found in the anterior region (64%). Additionally, in both regions, units responding to different body parts other than the head were often found adjacent to each other. Thus, there was only a rough somatotopy, with the majority of face units located anteriorly but with considerable convergence for all other parts of the body surface.

Because the units in the granular insula, like those in visual area TE, are modality specific and have bilateral receptive fields, our data are consistent with the proposal that Ig serves as a critical link in a somatosensory-limbic pathway much as area TE does for the visual-limbic pathway.

V. Electrophysiological studies of SII neurons in animals with SI ablations.

We have examined the responses of SII neurons to somatic stimulation in monkeys anesthetised lightly with nitrous oxide. In intact animals, SII neurons respond to light tactile stimulation of small, circumscribed receptive fields located predominantly on the contralateral side of the body. In animals chronically prepared with ablations of SI that spared only portions of the head and foot representations, the vast majority of SII neurons no longer responded to somatic input.

In two monkeys with SI ablation combined with forebrain commissurotomy, 50 penetrations were made into the region of the lateral sulcus in which SII is located. In those penetrations 179 single units or multiunit clusters were isolated and studied for their responsiveness to tactile stimulation. Units in three of these sites responded to tactile stimulation of the head or face and eight responded to stimulation of the hand or arm. Of those responding to forelimb stimulation only four responded to light tactile inputs. By contrast, in six normal animals in which recordings were made to locate sites for injections of anatomical markers, 109 of 119 recording sites in SII responded to tactile stimulation. Of the 75 of these units that could be clearly classified as responding to either light cutaneous or deep stimulation, 55 responded to light tactile inputs. In a third monkey with SI ablation whose forebrain commissures were left intact, 43 of 129 recording sites had units that responded to tactile stimulation. The units in the rest of the sites could not be driven. Of the 43 responsive units only 19 responded to light tactile stimulation and only 4 of those were located on the hand or arm.

These data suggest that the input SII receives from SI is necessary for the responsiveness of SII units to light tactile stimulation. This conclusion is consistent with the hypothesis, generated on the basis of the differential laminar patterns of termination of the reciprocal SI-SII projections, that the primary flow of information about sensory input is from SI to SII and that these two fields, at least, are organized in a hierarchical manner.

VI. Studies on the projections of cortical somatic fields in the cat.

In order to obtain data concerning the organization of the cortical somatosensory fields in a second species, and to begin an examination of sensorimotor relations, injections of tritiated amino acids were made into areas 4, 5, and SIV of the cat. As a first step, projections from these cortical areas to the brain stem were examined. The results demonstrate a set of parallel pathways to several regions of the mesencephalon and pons.

After injections into area 4, the magnocellular portion of the red nucleus (RNmc), the intermediate and deep layers of the superior colliculus (SC), and the ventral portion of the periaqueductal gray were labelled in the mesencephalon. More caudally, label was found in the tegmental reticular nucleus, central tegmental fields, and vestibular nuclei. Area 5 injections produced labeling primarily in RNmc, with an additional, smaller projection to the intermediate layers of the SC. Injections into SIV labeled primarily the

intermediate and deep layers of the SC, with additional labeling in the ventral pontine nuclei.

The patterns of labeling in the RN appeared similar after area 4 and area 5 injections, while the patterns of labeling in the SC appeared similar after area 4 and SIV injections. Thus, area 5 and SIV each appear to duplicate one portion of the motor cortical output arising in area 4.

SIGNIFICANCE TO MENTAL HEALTH RESEARCH:

Previous studies concerning the connections of the somatosensory system have been relatively restricted in scope. This project supplies the first comprehensive look at the entire somatosensory system and how it may connect with the limbic structures necessary for memory. Furthermore, this project is yielding fundamental insights into how the cerebral cortex processes information by describing the precise laminar pattern of connections and by adding new data about the thalamic connections of these fields. As a whole, our studies have demonstrated remarkable parallels between the organization of the somatosensory and the visual systems, suggesting that common mechanisms of perception and memory operate within both, and that further studies of each one will illuminate the other.

PROPOSED COURSE OF RESEARCH:

The anatomical tracing experiments have now been completed and the results are being prepared for publication. The physiological recordings of insular neurons will continue in order to expand our sample size and to examine more anterior portions of the insula. Additional animals will be prepared with SI lesions to replicate and extend our preliminary findings and to examine other somatosensory areas for their dependence on SI.

NOTICE OF INTRAMURAL RESEARCH PROJECT

201 MH 02038-02 LN

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Ontogenetic development of memory and habit formation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

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TOTAL MAN-YEARS.

2.0

PROFESSIONAL:

1.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Memory formation and habit formation are two qualitatively different retention processes based on separate neural mechanisms. Recent developmental studies in monkeys have indicated that these two systems of retention are developmentally dissociable, with the habit system maturing earlier than the memory system. On the evidence that the limbic memory system is not fully developed in infants, we have begun to prepare monkeys with neonatal removal of this system to see how cognitive, emotional, and social behavior develops in animals whose global amnesia might persist from infancy through adulthood. Animals with neonatal removal of area TE, a higher-order station of the visual system, are serving as controls. Preliminary results indicate that at two and six months of age, monkeys with neonatal limbic lesions display abnormal social behavior, whereas the operated controls are essentially unimpaired relative to normal infants. Examined at three months of age, animals with neonatal ablation of area TE showed a transient impairment of habit formation (compared to permanent impairment in adults with the same lesion), whereas the habit formation of animals with neonatal limbic lesions was intact (just as in adults). Interestingly, data on both normal and operated infants are suggestive of sexual dimorphism in the development of the habit system. At ten months of age, the animals with neonatal limbic lesions are markedly impaired in memory formation (just like adults), whereas the operated controls showed significant functional sparing of memory (compared with adults). Our tentative conclusion is not only that early and late brain damage have different consequences but also that the direction of the difference depends on the locus of the lesion (cortical or subcortical), the type of retention process examined (memory or habit), and the sex of the subject.

PROJECT DESCRIPTION

Findings from studies of the effects of lesions in adult monkeys suggest that memory and habit formation are qualitatively different retention processes based on separate neural mechanisms. The memory system, which serves both recognition and associative memory, utilizes a cortico-limbo-diencephalic circuit. By contrast, the habit system, which mediates retention of stimulus-response connections, probably depends in large part on a cortico-striatal system. Our recent studies of behavioral development in infant monkeys have suggested that these two systems are developmentally dissociable, in that the nonlimbic habit system appears to mature considerably earlier than the limbic memory system. On the evidence that the limbic memory system is essentially nonfunctional in infants, we have begun to prepare monkeys with neonatal removal of this system in an attempt to see how cognitive, emotional, and social behavior develop in animals whose global amnesia might persist from infancy through adulthood. In addition, to reevaluate whether visual recognition memory does indeed appear only late in ontogenetic development, we have begun to measure the memory ability of infant monkeys using the preferential-viewing task, a task widely used to study recognition memory in human infants. In tandem with these developmental studies of behavior, we have begun to map the distribution of opiate receptors in the brain of the newborn monkey.

Experiment 1:

Infant rhesus monkeys received damage to either the limbic system, i.e. amygdalo-hippocampal complex, or the anterior part of inferior temporal cortex, i.e. area TE. Both lesions are known to produce severe impairment of visual memory in adult monkeys. The bilateral lesions were performed in two unilateral stages at approximately one week and three weeks of age, respectively. Three factors dictated selection of the TE lesion as the control operation: (a) whereas amygdalo-hippocampal removals in adult monkeys impair the ability to form new memories but not the ability to acquire new habits, TE lesions in adults produce a severe impairment in both forms of retention; (b) conversely, disturbances in social behavior have been observed after limbic but not after TE lesions in adulthood; and (c) since both area TE and amygdalo-hippocampal lesions produce impairments in visual memory, the use of these two types of ablation permit comparison between the effects of neonatal cortical versus neonatal subcortical lesions on the development of visual memory. Each experimental and operated control animal was age-matched with a normal monkey. We plan to follow the behavior of these animals from birth to five years of age in order to assess the effects of their neonatal lesions on (1) the maturation of both cognitive functions and skill learning, as measured by a variety of visual-memory, problem-solving, and habit-formation tasks, and (2) the development of emotional and social behaviors, as measured by interactions with familiar vs. unfamiliar and normal vs. operated monkeys of both sexes and various ages, and by reactions toward familiar vs. unfamiliar and emotionally neutral vs. emotionally challenging environments and stimuli. To date, we have operated eight monkeys with bilateral amygdalo-hippocampal lesions and eight with bilateral TE lesions.

These monkeys were age-matched with thirteen normal animals and have already undergone some testing for social behavior and learning abilities.

At two months of age, five animals with limbic lesions (3 females and 2 males) and six with TE lesions (3 females and 3 males) were tested for social behavior in two different situations: (a) the operated animal and its age-matched normal control were caged separately but adjacently so that the two monkeys could reach and touch each other; and (b) the two monkeys were placed together in a test cage containing plastic toys and towels. In the first situation, the infants with limbic lesions displayed a greater frequency of stereotyped behavior and self-directed activity than the normal controls. In addition, they showed suppressed exploration of their surroundings and reduced social interaction with the normal animal in the adjacent cage. The infants with TE lesions, by contrast, displayed no disturbances in social behavior and interacted normally with the unoperated controls. They were generally the most active, however. In the second situation, preliminary results suggest that infants with limbic lesions again display more disturbed behaviors and less social interaction than either control group. More information is needed, however, before a firm conclusion can be reached, since these disturbances seem to vary according to the sex of the monkeys in the social group and the total amount of time the two monkeys of a pair have previously spent together.

At three months of age, the animals were trained in a concurrent object discrimination learning task with 24-hour intertrial intervals, a sensitive measure of habit-formation ability. We have previously reported that unlike animals given TE lesions in adulthood, who show a severe deficit in acquiring the task, the infants with TE lesions were only slightly impaired. With more animals now added in the study, it appears that the effect of neonatal TE lesions on the habit system is more complex than originally thought. When the learning scores were analyzed according to sex, interesting differences were found. Unlike normal adult males who performed as efficiently as normal adult females, the male infants required more days of training to reach criterion than did the female infants. In fact, normal female infants performed almost as well as both female and male adults. On the other hand, whereas female infants with TE lesions performed significantly worse than the female controls, the male infants with TE lesions did not differ significantly from the normal male infants. The data thus suggest that TE lesions in infancy affect females more than the males, perhaps because at that age area TE is more fully developed in the females than in the males. By contrast, limbic lesions in both adults and infants of both sexes appear to leave habit formation intact. The data on both normal and operated infants are thus suggestive of sexual dimorphism in the development of the habit system.

At six months of age, these animals are undergoing additional testing for social interactions in two different situations: (1) the same age-matched operated and normal monkeys tested together early in life are paired in a test cage; and (2) both the operated and normal monkeys are paired with a completely unfamiliar age-matched normal monkey. Interestingly, whereas the animals with limbic lesions tested so far displayed only moderate reduction of

social contact when observed with their familiar normal control, their initiation of social play was almost abolished when they were paired with the unfamiliar control. This lack of social contact was not evident in the young animals with TE lesions, who, like normals, constantly engaged in play and other forms of social interactions with both familiar and unfamiliar monkeys. Although the results are preliminary, they strongly suggest that limbic lesions in infancy lead to marked impairment in social development.

At 10 months of age, the animals are undergoing testing in a one-trial visual recognition task, a sensitive measure of memory formation. Like animals given limbic lesions in adulthood, those with neonatal limbic lesions demonstrated a severe impairment in recognition memory. However, unlike animals given TE lesions in adulthood, who show a profound loss of recognition memory, the animals given neonatal TE lesions were nearly unimpaired. The small number of animals in each group tested to date have not allowed a comparison between males and females. With this proviso, our findings suggest that although area TE is involved in memory formation in adults, neonatal ablation of this cortical area leads to a significant functional sparing of the ability; in contrast, neonatal limbic ablation leads to virtually the same impairment of the ability as limbic lesions in adulthood.

The evidence suggests that the consequences of early temporal-lobe damage are different from those of later damage and, further, that the direction of the difference depends on whether the locus of injury is cortical or subcortical, whether the lesion is made in a male or a female, and whether the task measures habit or memory formation.

Experiment 2:

Our evidence on the development of habit and memory formation in infant monkeys suggested that, when measured by problem-solving ability, visual recognition memory develops late in ontogeny. In the present study, we traced the development of visual recognition in infant monkeys as determined by preferential viewing of novel stimuli, a measure widely used to study visual recognition in human infants. In addition, to test whether the recognition ability measured by the preferential viewing task is mediated by the memory system or the habit system, we have begun to present this task to infants that have received neonatal limbic lesions.

Normal infants and infants with neonatal limbic lesions from Experiment 1 were studied, as well as normal and limbic-operated adults. Performance on the preferential viewing task, which measures how fixation time is distributed between a familiar and a novel visual stimulus, was assessed in the infants periodically at ages 5, 15, and 30 days and in the adults at the age of 3-4 years. The results indicate that normal infants of 15 and 30 days of age, like normal adults, spend more time fixating novel than familiar stimuli. By contrast, 5-day-old normal infants do not. The visual preference for novelty develops gradually and becomes stronger with increasing age, being strongest in the adults. By contrast, the preference was completely absent in 15-day-old infants with unilateral limbic lesions, 30-day-old infants with bilateral limbic lesions, and adults with bilateral limbic lesions. These

preliminary findings indicate that preferential viewing of novel stimuli is based on a primitive recognition process, present in rhesus monkeys by at least 15 days of age, and dependent on the integrity of limbic structures.

Experiment 3:

Opiates have been shown to play a role in memory formation, and the recent development of autoradiographic receptor-binding methods has indicated that in adult monkeys, opiate receptors are distributed densely in structures intimately involved in memory processes. Because certain forms of memory develop late ontogenetically, we have started to trace the ontogenetic development of opiate-receptor binding sites in the infant monkey brain. In our first attempt, the distribution of opiate receptors in the brain of a newborn rhesus monkey was mapped by in vitro autoradiographic localization of [³H] naloxone. The methods are outlined in another project from this laboratory (MH 02040-01). The autoradiographs at selected levels through the newborn brain were compared to those from two adult monkey brains that had been processed in the same way. The comparison revealed that the adult distribution of opiate-receptor binding was already present at birth in allocortical areas and the hippocampal formation. These primitive areas showed conspicuously higher levels of opiate binding than the neocortex in both the newborn and adult brains. In the neocortex, by contrast, differences between the infant and adult brains did appear. For example, whereas the adult brain was characterized by areal specific laminar patterns in primary sensory, motor and premotor, and dorsolateral prefrontal areas, the newborn brain was not. In the newborn brain, only the primary visual cortex had a distinct laminar pattern of labeling; in all other neocortical areas the infragranular layers showed relatively high levels of opiate-receptor binding. In the adult brain, polysensory areas (e.g. PG and TF) showed greater labeling density than modality-specific sensory areas, but this density difference was not apparent in the newborn brain. At the subcortical level, the density and pattern of opiate receptors in the infant and adult brains were similar, with a patchy mosaic of labeling in the striatum, high levels of labeling in certain amygdaloid and thalamic nuclei, and an absence of labeling in the mamillary bodies.

The findings suggest that the distribution of opiate receptors in the macaque brain is adult-like at birth in limbic and other subcortical structures but is not yet fully developed in neocortical areas.

SIGNIFICANCE TO MENTAL HEALTH RESEARCH:

Developmental studies of the effects of early brain damage are of great importance for the assessment and understanding of those errors of central nervous system maturation that cause children to become autistic, dyslexic, learning disabled, or mentally retarded. This project will supply the first comprehensive investigation of social and cognitive development of monkeys suffering from an amnesia induced early in infancy. It will thus permit comparisons of the cognitive and social behavior of the neonatally operated animals with those of animals who have sustained the same lesion in adulthood i.e. after memories have been formed and consolidated in cerebral tissue

outside the limbic system. In addition, comparison of the effects of early cortical and subcortical lesions will help answer whether or not compensatory mechanisms always operate to promote recovery from early brain injury. Our preliminary results suggest otherwise. Finally, in assessing the effects of early and selective temporal-lobe damage on infant, juvenile, and adult behavioral patterns, this project will help to evaluate two provocative proposals from the clinical literature: (a) that early dysfunction of the limbo-thalamic memory system is one cause of childhood autism, a syndrome characterized by dramatic social and emotional disturbances not seen in adults with the same neuropathology; and (b) that the reason a pure case of anterograde amnesia like the one seen in adults has never been reported in a child is that the clinical picture of an amnesic child, being overlaid with autism, is entirely different from the clinical picture of an amnesic adult. Furthermore, the discovery that the visual preference for novelty is a primitive recognition process mediated by the limbic system provides new insight into the normal development of memory processes; specifically, it helps identify the maturational sequence of limbic-dependent memory abilities.

PROPOSED COURSE OF RESEARCH:

Our plan is to continue with the examination of the effects of neonatal limbic lesions in monkeys on social and emotional behavior as well as on memory and learning at several periods throughout development from infancy to adulthood in order to test whether such a preparation does indeed provide an animal model of childhood autism. Having discovered that development of the habit system is sexually dimorphic, we shall initiate studies to determine the neuroendocrinological substrate of this dimorphism. Having discovered also that one form of limbic-dependent memory develops early while another develops only later, we shall attempt to trace this maturational sequence more fully. The ultimate aim of such experiments will be to determine whether the phenomenon of infantile amnesia is due to the absence of a fully functional memory system in early childhood. We shall also continue our attempts to follow the ontogenetic development of opiate receptors in infant monkeys.

PUBLICATIONS:

Bachevalier, J. and Mishkin, M. An early and a late developing system for learning and retention in infant monkeys. Behav. Neurosci. (in press) 1984.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02039-02 LN

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cholinergic mechanisms in memory

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TOTAL MAN-YEARS:

1.5

PROFESSIONAL:

1.0

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The basal forebrain cholinergic system consists of the nucleus basalis of Meynert, the medial septum and the diagonal band of Broca. The first of these nuclei provides the major source of cholinergic input to the cortex and amygdala, whereas the others provide the cholinergic input to the hippocampus. Dysfunction of this cholinergic system has been proposed as one explanation for the memory impairments observed in Alzheimer's disease. To assess the contributions of these cholinergic areas to recognition memory, we compared the effects of neurotoxic lesions of nucleus basalis alone, medial septum plus diagonal band, or all three regions combined. The results suggest that combined damage to all three is necessary to produce significant impairment in recognition memory in monkeys, perhaps because only such damage causes cholinergic denervation of both the amygdala and the hippocampus. In a separate study in normal monkeys, we found that the cholinergic antagonist scopolamine, in a dose range that impairs recognition memory, has no effect on habit formation, as measured by an object discrimination task involving 24-hour intertrial intervals.

PROJECT DESCRIPTION:

Other projects in this laboratory dealing with memory processes in monkeys have revealed some of the cerebral areas that are required for specific forms of memory. We are now attempting to characterize the neurotransmitters and neuromodulators that are involved in these particular forms of memory by administering various pharmacological agents to normal animals and to animals with lesions of critical cerebral structures. Our ultimate experimental goal is to measure the effects of discrete injections of drugs made into selected cerebral areas while the monkeys are performing a variety of memory tasks. As a first approximation to this goal, we have begun to test the effects of peripherally administered drugs on these same tasks.

Experiment 1:

Our previous work indicated that the cholinergic drugs scopolamine and physostigmine produced impairments and improvements, respectively, in recognition memory in rhesus monkeys. In order to help determine whether this effect of cholinergic drugs was specific to memory, we studied the effects of the drugs on habit formation. Earlier studies from the laboratory had demonstrated that whereas monkeys with limbic (i.e. amygdalo-hippocampal) lesions are severely impaired on a recognition memory task such as delayed nonmatching-to-sample (DNMS), they can nevertheless learn to discriminate a large set of object-pairs even when the set is presented only once every 24 hours (24-hour concurrent learning). This nonlimbic learning process has been characterized as habit formation. To determine if cholinergic mechanisms are also involved in habit formation, we administered scopolamine, a muscarinic receptor antagonist, to three animals trained in the 24-hour concurrent learning task. First, one set of 20 object-pairs was presented for learning without drug, and then the effects of scopolamine (10 ug/kg) or saline were assessed on the learning of three subsequent sets. This dose of scopolamine had previously been shown to produce a 50% increase in errors in the DNMS task. A counter-balanced design was used to control for factors such as the development of tolerance to the effects of repeated administration of the drug. One animal received the drug before the session, one animal received the drug after the session, and the third animal received saline. These treatments were changed with each new set of object-pairs, so that each animal received each treatment condition. Scopolamine, administered either before or after the session, failed to affect the rate at which the monkeys learned the sets of object discriminations. After the animals had learned the final set, a dose-response curve was determined for the effect of scopolamine on performance with this set. Even with extremely high doses (178 ug/kg), the monkeys were able to perform the task correctly. These results indicate that (a) cholinergic mechanisms are much less important for habit than for memory formation, and (b) the effect of cholinergic drugs on performance of the memory task cannot be attributed to an effect on either perception, attention, motivation, or some general learning ability. To examine the possibility that the effects on habit formation were negative because of a slowly developing tolerance to repeated administration of scopolamine, we have begun a replication of the experiment using monkeys that have had no previous exposure to the drug.

The effects of scopolamine are also being examined on associative memory. Three cynomolgus monkeys performing a cross-modal (touch to vision) version of delayed nonmatching-to-sample have been found to be severely impaired with administration of 32-56 mg/kg of scopolamine, doses much lower than those that had no deleterious effect on habit formation. Thus, associative memory, like recognition memory, appears to be sensitive to manipulation of the cholinergic system, a finding that provides support for the idea that both forms of memory depend on the same basic mechanism. Further research will examine whether scopolamine affects the intramodal versions of delayed nonmatching-to-sample.

Experiment 2:

In Alzheimer's disease, which is often accompanied by memory loss, the most severely affected cell groups are located in the basal forebrain cholinergic system, which includes the nucleus basalis of Meynert, the medial septum, and the diagonal band of Broca. The first of these areas is known to provide the major cholinergic input to the cortex and amygdala, while the other two provide the cholinergic input to the hippocampus. In an earlier study, we found that although lesions of nucleus basalis alone did not produce impairments in recognition memory, they did cause an increased sensitivity to the effects of scopolamine on memory, an effect that was correlated with the size of the nbM lesion and the consequent reduction in activity of cortical choline acetyltransferase (ChAT), a major enzyme in the synthesis of acetylcholine. To pursue this lead, we next examined the contributions of the medial septal and diagonal band nuclei to recognition memory in monkeys by damaging these two areas both alone and in combination with nucleus basalis, using the neurotoxin, ibotenic acid. When compared to unoperated controls, only the animals with combined lesions of all three areas were impaired in recognition memory. These animals not only required more trials than controls to relearn the recognition task but also showed significant losses as the memory demands of the task were increased. The results strongly support the view that the basal forebrain cholinergic system is important for memory. We are currently examining the effects of scopolamine and physostigmine in these animals. In addition, we will soon initiate a new project to determine if discrete lesions in nbM will produce selective impairments in associative memory that can then be correlated with decreased ChAT activity in the specific cortical areas denervated by the lesion.

Experiment 3:

Administration of the narcotic antagonist naloxone has been reported to produce improvements in memory and cognitive functioning in some patients with Alzheimer's disease. Recent evidence also suggests that naloxone can facilitate performance in a spatial memory task in rats. To determine if opioid systems are involved in memory processes in monkeys, we administered the opiate antagonist naloxone (0.1, 0.3, 1.0, 3.2, or 10.0 mg/kg) to five macaques trained in delayed nonmatching-to-sample. In each of the five animals, the percentage of correct choices was significantly increased above control levels following a dose of 0.3 mg/kg. Higher doses yielded variable effects, producing either no change or even impairments in performance. The results indicate that selective doses of naloxone can produce reliable, though

modest, improvements in recognition memory in monkeys. To examine the functional selectivity of the improvement, we are testing the effect of naloxone on habit formation with post-session injections of 1 mg/kg naloxone (or an equal volume of saline) during learning of the 24-hour concurrent learning task described earlier.

Experiment 4:

There are a number of anecdotal reports suggesting that chronic marijuana use has a detrimental effect on short-term memory in humans. As part of a collaborative project with the National Institute of Drug Abuse, we administered Δ -9 tetrahydrocannabinol (THC), the active constituent of marijuana, both intramuscularly and orally, to three rhesus monkeys trained in recognition memory. The effects of intramuscular injections of THC were highly variable, producing inconsistent effects on performance of the task. With oral administration, by contrast, the higher doses of THC produced consistent recognition impairments in all animals. We have now initiated a follow-up study to determine if 30 days of chronic oral administration of THC produces impairments which persist beyond the period of drug administration.

SIGNIFICANCE TO MENTAL HEALTH RESEARCH:

One goal of our pharmacological and neurotoxic-lesion studies is to produce an animal model of the memory dysfunction associated with Alzheimer's disease. Such a model is important not only for understanding this disease process and others accompanied by memory loss and dementia but also for understanding the mechanisms underlying normal memory and normal cognitive function. Furthermore, an animal model is of inestimable value in any attempt to evaluate therapeutic agents. Our results to date suggest that interference with central cholinergic mechanisms either with drugs or with lesions of the basal forebrain system produce behavioral deficits that are qualitatively similar to those observed in early stages of Alzheimer's disease. Our studies with naloxone in normal animals suggest that this drug may have potential therapeutic applications. Our continuing studies with THC represent an attempt to identify and characterize potential detrimental effects of this commonly abused drug. Intensive pursuit of these lines of inquiry should yield important insights into the neurochemistry of storage and retrieval processes, which are basic to all mental functions.

PROPOSED COURSE OF RESEARCH:

Our results to date support the view that central cholinergic mechanisms play an important role in memory. We are now in the process of determining the specificity of that role by testing the effects on recognition memory of drugs that act through other neurotransmitter systems and by testing the effects of cholinergic drugs on forms of memory other than recognition. Based on the results of these pharmacological experiments, we will soon begin examination of the effects of direct injection of the same drugs into cerebral sites selected on the basis of findings from our lesion studies. In addition, we are attempting to make more discrete lesions than before in nucleus basalis with the goal of producing selective impairments in associative memory and

correlating these impairments with decreases in enzyme activity in the denervated cortical areas. We will also continue to analyze the actions of drugs such as physostigmine and naloxone for potential therapeutic effects. Finally, we will continue to study the consequences of THC administration, both acutely and chronically, on memory and cognitive processes in monkeys.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02040-01 LN

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Functional analysis of neurotransmitter systems

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

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TOTAL MAN-YEARS:

0.5

PROFESSIONAL:

0

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Neurotransmitter systems have been implicated in many higher-order functions, both cognitive and emotional, but specific sites and mechanisms of action have not been identified. Studies to localize specific receptors for certain neurotransmitters in the monkey have been undertaken to attack these problems. Findings following lesions of the amygdala suggest that there may be opiateergic projections from the amygdala to higher-order sensory processing areas, such as the anterior insula and orbitofrontal areas. Developmental studies show that whereas limbic cortical areas and most subcortical regions have adult-like patterns of opiate receptors at birth, neocortical areas have simplified and undifferentiated binding patterns unlike the patterns seen in adults. Metabolic studies link the level of mu opiate receptors to the rate of protein phosphorylation in the F1 band. Since phosphorylation of F1 protein has been correlated with learning, these results suggest that opiates may help control the learning-related phosphorylation process.

Autoradiographic localization of benzodiazepine and beta-carboline receptors in monkey show that both drugs bind with apparently identical distributions, implying that both act on the same brain regions to produce their effects on anxiety.

PROJECT DESCRIPTION:

The limbic and diencephalic areas found in other projects of this laboratory to be critical for learning and memory contain high levels of opiate receptors, and it is now known that opiates may alter learning ability under a variety of circumstances. The pathways by which opiate-containing neurons may project to the cortex are still unknown, however, as are the specific pre- and post-synaptic effects of endogenous opiates. The initial stages of this project will attempt to (I) describe the opiate pathways that may affect learning, (II) relate the level of opiate-receptor binding to learning-related protein phosphorylation, (III) describe the distribution of opiate receptors in developing brain that may account for the developmental increases in perceptual and cognitive ability, and (IV) examine the distributions of receptors for benzodiazepines and beta-carboline that may account for the action of these drugs in decreasing and increasing anxiety.

Methods Employed:

Receptor binding and *in vitro* autoradiographic techniques are used to localize and quantify receptors. After sectioning unfixed monkey brains on a cryostat, the sections are thaw-mounted onto slides and incubated in tritiated ligands for various receptors and processed for autoradiography. Metabolic studies are performed on homogenized monkey brain tissue that has been dissected according to functional and cytoarchitectonic criteria and then assayed for receptor-binding levels and for the rate of learning-related protein phosphorylation. Correlations are then performed between the levels of mu-receptor binding and protein phosphorylation.

Major Findings:

I. Opiatergic Projections of the Amygdala

Analysis of [³H] naloxone binding levels has been performed on sections from one monkey brain that had received a unilateral amygdectomy 30 days prior to sacrifice. The sections show an increase in naloxone binding ipsilateral to the lesion in the anterior portions of the insula, which projects to the limbic areas of the temporal lobe, and in regions of the orbitofrontal cortex; all of these areas appear to be essential for memory formation. This increased binding is interpreted as a denervation supersensitivity of mu receptors following amygdectomy, and suggests that the amygdala sends an opiate projection to the cortical regions mentioned. We are now attempting to replicate this finding in additional monkeys, extend the analysis to other regions of the brain, and examine kappa and delta as well as mu receptor subtypes.

II. Metabolic Studies

In order to correlate the levels of opiate binding with levels of learning-related protein phosphorylation, correlations of naloxone binding levels and protein phosphorylation rates have been carried out in two monkeys. The results demonstrate: (a) an inverse correlation across 22 brain

regions between the density of [^3H] naloxone binding sites and the in vitro incorporation of phosphate into the 45kD band, Fl ($r=0.61$; $p<.01$); (b) the presence of a 47kD protein with properties like those of the 45kD band in regions of highest naloxone binding; and (c) a positive correlation between the phosphorylation of a 49kD protein and [^3H] naloxone binding levels ($r=0.56$; $p<.01$). These findings suggest a local control of protein phosphorylation in monkey cerebral cortex corresponding to opioid receptor levels and indicate that opioid peptides may help control metabolic processes at the synaptic level that underlie memory formation.

III. Developmental Studies

Because certain forms of memory develop late during maturation, we have started to trace the development of opiate receptor binding sites in the brain of infant monkeys. In our first attempt, the distribution of opiate receptors in the brain of a newborn rhesus monkey was mapped by in vitro autoradiographic localization of [^3H] naloxone. The results, which are described fully in another project from this laboratory (MH 02038, Experiment 3), show that whereas subcortical and limbic structures have adult-like patterns of naloxone binding, neocortical areas have an immature pattern. This pattern is nearly identical across all neocortical areas and is characterized by a lack of the laminar-specific distribution of receptors seen in adults.

IV. Localization of benzodiazepine and beta-carboline binding sites

Although biochemical evidence suggests that benzodiazepine (BDZ) and beta-carboline (BC) binding sites are part of the same supramolecular complex, there has not yet been an anatomical demonstration of this receptor localization. To gather such evidence, we performed an in vitro autoradiographic mapping study of the distribution of [^3H] flunitrazepam (FLN) and [^3H] BC binding in a rhesus monkey brain.

The major finding is that, in the monkey, specific FLN and BC binding sites have apparently identical distributions. Limbic structures such as the amygdala and the hippocampus, which have been implicated in the mediation of anxiety, were differentially labeled in identical fashion by the two ligands. The lateral, accessory, and cortical nuclei of the amygdala were heavily labeled by both. Also, there was intense labeling by both in the molecular layer of the hippocampus and in regions related to the hippocampus, such as the medial portion of the medial mamillary nucleus and the lateral dorsal and anterior nuclei of the thalamus. Furthermore, the subfields of the hippocampal formation could be distinguished on the basis of differential labeling densities of both ligands. By contrast, the locus coeruleus and the raphe nuclei, which have also been implicated in anxiety, could not be identified on the basis of labeling densities of either ligand.

The apparently identical patterns of the BDZ and BC binding suggest that these two agents are acting on the same, or closely linked, receptors. The location of especially dense labeling in certain limbic structures supplies additional evidence that these structures are involved in the modulation of anxiety.

SIGNIFICANCE TO MENTAL HEALTH RESEARCH:

Knowledge about the functions of neurotransmitter systems has both basic and applied value. The studies described here represent one of the few attempts to study these systems in primates. Descriptions of the opiateergic pathways of the forebrain will supply the fundamental descriptive information needed to understand the role of opiates in higher-order behavioral functions and will help guide neurobehavioral experiments by supplying targets for lesion experiments. The studies of protein phosphorylation may supply information concerning the mechanisms by which the opiates affect neuronal metabolism in general and learning specifically. Eventually, this may lead to therapeutic advances in the treatment of memory and learning disorders.

Localization of benzodiazepine and beta-carboline binding sites will supply descriptive information suggesting where their effects on anxiety may be mediated.

PROPOSED COURSE OF RESEARCH:

Our major goals for the coming year are to replicate the findings in additional animals and to extend them by examining receptor subtypes. Specific aims for each of the four experiments are as follows: (I) examine additional brain regions that receive amygdaloid inputs and examine changes in mu, kappa, and delta receptor binding by using ligands that are highly specific for each; (II) further characterize the F1 protein by means of 2-dimensional gels, examine the activity of the specific kinase that phosphorylates F1, and quantify the F1 content in various regions of the monkey brain; (III) replicate the finding in the neonate with an additional animal and to examine the distribution of mu, kappa, and delta receptors; (IV) replicate and quantify the initial findings on benzodiazepine and beta-carboline binding and determine whether amygdectomy has an effect on such binding. (V) An additional study is planned to map the distribution of muscarinic and nicotinic cholinergic receptors in both the adult and infant rhesus monkey brain.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00471-29 LPP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of Heredity and Environment in Schizophrenia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

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TOTAL MAN-YEARS:

1.5

PROFESSIONAL:

1.0

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

 (a) Human subjects (b) Human tissues (c) Neither (a1) Minors (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The project is composed of the following studies: (1) An intensive multi-disciplinary study of a family with MZ quadruplets (daughters) concordant as to schizophrenia but discordant as to severity and outcome. (2) Studies of adoptees and their biological and adoptive families. (3) A study of children (of schizophrenic and control parents) reared in town or kibbutz in Israel.

Project Description

The project is composed of the following studies: (1) An intensive multi-disciplinary study of a family with MZ quadruplets (daughters) concordant as to schizophrenia but discordant as to severity and outcome. We are continuing our contacts with this family to see what happens in the clinical course of these women and to see how the course is related to earlier and to current life experiences; (2) Studies of adoptees and their biological and adoptive families in Denmark; (3) A study of children (of schizophrenic and control parents) reared in town or kibbutz in Israel.

The objectives of this project are to understand how hereditary and environmental factors interact to make for schizophrenic outcomes of varying types and degrees.

1. The Genain Quadruplets

In 1963 David Rosenthal published the results of an extensive study of a group of four women, identical quadruplets, all of whom had succumbed to schizophrenic illness at some point during their late teens or early twenties. The women (who were named by Rosenthal for this publication: Nora, Iris, Myra, and Hester, i.e., N.I.M.H.) were studied by a group of psychologists and psychiatrists at the NIMH and were examined with virtually all of the methods extant in the late 1950's for studying schizophrenia and psychological deficit. After a period of study at the NIMH which extended over several years and which relied heavily on psychiatric treatment of the dynamic variety (both as therapy and as a means of gaining information), Rosenthal summarized the investigative effort in the Genains by the suggestion that the diathesis-stress theory was a reasonable way of accounting for the differences in the severity of their psychiatric illness. Although they shared an identical heredity (diathesis), differences in the way they were treated by their parents and significant others in their environment led to different expectations and self-pictures and consequently to different phenotypic expression of the schizophrenic disease. The more competent "pair", Nora and Myra, were more favored and fussed over; the smallest and least prepossessing physically, Hester, was most often bracketed with Iris. Willy-nilly, Hester and Iris were treated as the less competent and capable pair and more or less fulfilled that expectation. This is a somewhat oversimplified but reasonably accurate summary of the earlier view of the Genains.

Rosenthal and his early colleague and collaborator, Olive Quinn, maintained contact with the Genains and with their mother (who died last year at age 84). Through Rosenthal's and Quinn's good offices and contacts, we were able to persuade the Genain clan to return to NIMH for another period of study. In addition to the quadruplets themselves, the group included the mother, the husband of Myra (she is the only one to have married) and Myra's two adolescent sons. On this occasion, which lasted for a period of approximately 3 1/2 months, we tested the Genains with the full battery of neurobiological test procedures that have evolved over the last 25 years. The procedures included: an extensive series of genetic identity tests; biochemical determinations from blood, urine, and cerebrospinal fluid of various catecholamine compounds with emphasis on dopamine and norepinephrine; procedures related to the identification of possible

preexisting viral infection of the central nervous system; neuroradiological and neurophysiological tests (CT scan, PETT scan, evoked potential and EEG brain maps, brain stem evoked potentials); and an exhaustive battery of psychological and psychometric tests with a special focus on measurement of attention, arousal and memory. Two of the tests were essentially identical to measures employed in the late 50's--the continuous performance test and the reaction time paradigm. Further, for most of the behavioral tasks, we were able to examine the Genains both on and off medication--the latter after a period of at least two weeks free from the phenothiazine drugs they were taking on admission to the NIMH.

We conclude that the Genains are functioning about as well as they ever have in their adult lives, and scores on attention tests show improvement as compared with 1958 measures. This is likely attributable to the medication (primarily phenothiazines) and other supportive treatments they have received over the years. With respect to the varying degrees of illness seen in the Genains, the following findings appear relevant: the tests indicate that two of the women (Nora and Hester) deteriorated rapidly when removed from medication, and two (Iris and Myra) did not. The consequence of this is that the grouping of the quadruplets on the basis of their characteristics and abilities while they are medicated is different from that apparent while they are off medication. On medication the apparent pairing is Nora and Myra (as before) and Hester and Iris. Scrutiny of the test material, including the biochemical, physiological, neuroradiological and immunogenetic, as well as behavioral, leads to speculation that certain unique biochemical findings and differing types and amounts of cerebral pathology may constitute the fundamental cause of the variable expression of schizophrenia in the Genains. This set of circumstances is superimposed on a basic schizophrenic diathesis which is manifest in the biochemical and certain neurological and neurobehavioral findings. The interdisciplinary research effort represented by this series of studies is unique in the annals of schizophrenia research and has led, we believe, to testable hypotheses on the role of various neurobiological factors in the development, etiology, and expression of the disease.

A series of three studies with first authors respectively, Lynn Delisi, Monte Buchsbaum and Allan F. Mirsky, have been accepted for publication in Psychiatry Research and are in press at this writing.

2. The Danish Adoptee Study--Reanalysis of the Data

Using data from Danish health records, in a now-classic study, Rosenthal, Kety and Wender compared the frequency of schizophrenia spectrum disorders in two groups of persons adopted in infancy or early childhood: those with a psychotic parent (index group) and those whose biological parents had never had psychiatric treatment (control group). Significantly more disorder was found in the index than the control group. This study has been criticized recently on the grounds that subjects were included inappropriately (affective rather than schizophrenic diagnoses in the parents; insufficient information available about the father). We have completed a reanalysis of the original material using the new DSM III methods, and stricter exclusionary criteria applied to the parents. The results of the reanalysis yielded three times as many schizophrenia spectrum disorders in the index as in the control group, a slightly better result than that found in the

original Rosenthal et al., study. The difference between groups remains statistically significant, supporting the operation of genetic factors in the transmission of schizophrenia spectrum disorders. These have been published in the American Journal of Psychiatry. A second manuscript describing the relation (in these same subjects) between reported stress (in childhood) and severity of schizophrenia spectrum illness is under editorial review at this time.

(3) The Israel Kibbutz--High Risk Study

During the past year, the Laboratory has completed work begun in 1962 on the study of children at risk for schizophrenia in Israel, which was designed and initiated by Dr. David Rosenthal. The study has examined 100 children, of whom 50 had one schizophrenic parent, and 50 were born to two nonschizophrenic parents. Half of both "index" and control groups were reared in towns in traditional nuclear families, while the remaining half were reared in communal settings on kibbutzim.

Our work has been in two phases. The first has been to complete data analysis of the initial examination of subjects, done when they averaged 11 years of age, and a major portion of the second examination when they averaged 17 years of age, and prepare manuscripts for the first major publication of the results. It is not easy to present the problems involved in executing and completing a study involving this much international collaboration. There have been enormous technical difficulties in getting the manuscripts from the several Israeli collaborators, sending revisions back and forth and securing approval for various publication plans. This year (1984) the last of the manuscripts have been completed. At the present time, data analysis is complete, and the 18 manuscripts have been submitted for publication to the Schizophrenia Bulletin. In broad outline, the results indicate that index children were discriminable from controls in many areas of function, but kibbutz and town children did not differ on the experimental examinations. Furthermore, kibbutz versus town rearing had no discernible effect on the performance or behavior of high-risk children. Index children were found to be poorer in psychosocial adjustment, perform more poorly in school, manifest a number of neurological "soft signs", and show deficits on psychological tests requiring high levels of attention, visual integration, and visuo-motor coordination. An important negative finding was lack of differences between index and control children on psychophysiological measures of arousal and habituation in the first examination. Recent information (which will not be included in the Schizophrenia Bulletin publication) indicates that the lack of psychophysiological difference was sustained in the second examination.

The second phase of the study has involved the collaboration of Dr. Arje Latz, of Boston University, who has been engaged in conducting follow-up interviews with study subjects. These subjects are now in their mid-twenties, at the peak of their risk period for schizophrenic breakdown. Ninety of the surviving 99 subjects have been seen. Results show that nine subjects fall within the "schizophrenia spectrum" (of whom six are DSM III schizophrenic), six from kibbutz backgrounds, and three from towns. When all DSM disorders are considered, more than five times as many ill subjects fall within the index (N=23) as within the control group (N=4). Furthermore, when schizophrenia itself is excluded, the remaining subjects with history of illness (including DSM III Major Affective

Disorder or Dysthymic Disorder) are found predominantly in the index-kibbutz cell (16 of the total of 27 in the cell). Other significant preliminary results include persistence of attention-related deficits in the index group, and continued poor social and work adjustment in high-risk subjects. A report of these findings, as well as other data describing the ill parents, will be included in the Schizophrenia Bulletin.

At present, work on the project centers around an attempt to identify "risk" factors in the offspring of schizophrenic persons.

Significance to Biomedical Research and to the Program of the Institute

The issue of the mode of heritability of mental illness, and factors which modify it, may be the highest priority of the Institute. This work contributes significantly to our knowledge in this area and ultimately, to our capacity to treat and prevent schizophrenia and related disorders.

Proposed Course

The completed manuscripts have been submitted and accepted for publication. It is estimated that an additional year or two will be necessary for the completion of this work, but the time table is not certain at this point.

Publications

Lowing, P.A., Mirsky, A. F. and Pereira, R.: The inheritance of schizophrenia spectrum disorders: A reanalysis of the Danish adoptee study data. Am. J. Psychiatry. 140: 1167-1171, 1983.

Mirsky, A. F., DeLisi, L.E., Buchsbaum, M.S, Quinn, O. W., Schwerdt, P., Siever, L., Mann, L., Weingartner, H., Zec, R., Sostek, A., Alterman, I., Revere, V., Dawson, S. D., Zahn, T. P.: The Genain Quadruplets: psychological studies. Psychiatry Research, in press., 1984.

DeLisi, L.E., Mirsky, A.F., Buchsbaum, M.S.: The Genain Quadruplets: 25 years later: A diagnostic and biochemical follow-up. Psychiatry Research, in press. 1984.

Buchsbaum, M., Mirsky, A.F., Delisi, L.E., Morihisa, J., Karson, C., Mendelson, W., King, A.C., Johnson, J., and Kessler, R.: The Genain Quadruplets: Electrophysiological, Positron-emission, and X-ray tomographic studies. Psychiatry Research, in press. 1984.

Mirsky, A.F., and Duncan-Johnson, C.: Nature versus nurture in schizophrenia--the struggle continues. Integrative Psychiatry. 2: 137-148, 1984.

Mirsky, A.F., Quinn, O.W., DeLisi, L., Schwerdt, P. and Buchsbaum, M. The Genain quadruplets-25 year follow-up of four monozygous women discordant for the severity of schizophrenic illness. Cohen, G. and Miller, N.E. (Eds.) Schizophrenia, Paranoia, and Schizophreniform Disorders in Later Life, in press, 1984.

Nagler, S. and Mirsky, A.F.: The Israeli Project: Introduction. Schizophr. Bull., in press, 1984.

Mirsky, A.F. The Israeli Project: Addendum: Bringing the Israeli High-Risk Study to Completion. Schizophr Bull., in press, 1984.

Nagler, S., Marcus, J., Sohlberg, S., Lifshitz, M., and Silberman, E.K. The Israeli Project: Clinical Observation of High Risk Children. Schizophr. Bull., in press, 1984.

Silberman, E.K., Magler, S., Ayalon, M., Shottan, J.H., and Meron, H. The Israeli Project: Clinical-Subjective Evaluation of High-Risk Children: Integration and Discussion. Schizophr. Bull., in press, 1984.

Silberman, E.K. and Tassone, E.P. The Israeli Project: The Israeli High-Risk Study: Statistical Overview and Discussion. Schizophr. Bull., in press, 1984.

Silberman, E.K. and Mirsky, A.F. The Israeli Project: The NIMH-Israel Schizophrenia Project: Comments on the Natural History of a Longitudinal High-Risk Study. Schizophr. Bull., in press, 1984.

18. Mirsky, A.F., Silberman, E.K., Latz, A., and Nagler, S. The Israeli Project: Adult Outcomes of High-Risk Children: Differential Effects of Town and Kibbutz Rearing. Schizophr. Bull., in press, 1984.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00484-24 LPP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Psychophysiological Responsivity and Behavior in Schizophrenia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Theodore P. Zahn, Ph.D. Research Psychologist LPP, NIMH

COOPERATING UNITS (if any)

Laboratory of Socio-Environmental Studies, Laboratory of Clinical Science, Clinical Neuropharmacology Branch, Neuroscience Branch, Biological Psychiatry Branch, and Adult Psychology Branch, NIMH

LAB/BRANCH

Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

1.7

PROFESSIONAL:

0.8

OTHER:

0.9

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The general purpose of this project is to investigate the roles of autonomic nervous system (ANS) activity, attention, and information processing and their interrelationships in the pathology, etiology, and prognosis of psychiatric disorders. A second purpose is to determine biological and psychological processes related to ANS activity. ANS activity is assessed by peripheral measures, such as skin conductance, heart rate, and skin temperature. Subjects are tested under conditions of rest, presentation of tones, and performance on reaction time, mental arithmetic, two-flash discrimination, or tachistoscopic recognition tasks. Biological mechanisms influencing ANS activity and attention are investigated by testing the effects of drugs and other treatments and by correlating these variables with enzyme activity, neuropeptides, and levels of biogenic amines and their metabolites. Psychological determinants are investigated by correlating the results with personality, mood, and personal history questionnaires by information from interviews, and by the effects of procedural variations.

Studies are being done on unmedicated patients with diagnoses of schizophrenia, affective disorder, obsessive compulsive disorder, anxiety-panic disorder, and autism to test the diagnostic specificity of patterns of ANS activity. Effects of state changes are studied in cases of multiple personality and in women in different phases of the menstrual cycle. Effects of pimozide, GHB, propranolol, prazosin, verapamil, and hemodialysis have been or are being studied in schizophrenics. Obsessive patients have been studied while taking clorgyline and clomipramine. The use of confirmatory factor analysis in data reduction and to improve quantification of ANS activity is being explored.

Others:

Carmi Schooler, Ph.D.	Senior Investigator	LSES, NIMH
Dennis Murphy, M.D.	Chief	CNB, NIMH
David Pickar, M.D.	Chief	SCS, NSB, NIMH
Thomas Uhde, M.D.	Staff Psychiatrist	BPB, NIMH
Thomas Robinson, Jr., Ph.D.	Guest Worker	LPP, NIMH
Daniel Hommer, M.D.	Staff Psychiatrist	NSB, NIMH
Judith Rumsey, Ph.D.	Staff Fellow	LCS, NIMH
Judith Rapoport, M.D.	Chief	SCP, LCS, NIMH
Thomas Insel, M.D.	Research Psychiatrist	CNB, NIMH
Frank Putnam, M.D.	Staff Psychiatrist	APB, NIMH

Project DescriptionA. Objectives

The major objective of this project is the further understanding of the role of autonomic nervous system (ANS) activity, information processing and attention, and their interrelationships in psychiatric disorders, primarily schizophrenia. The overall strategy involves studies of ANS and attentional relationships to diagnosis and prognosis, studies of the effects of drugs and other therapeutic interventions, "high risk" and personality studies in normal volunteers, and studies of the measurement of ANS activity.

B. Methods Employed

The general methods of these studies include measurement of ANS activity through skin conductance (SC) usually measured bilaterally, heart rate (HR), vascular activity (skin temperature and finger pulse volume), and respiration while subjects are resting, exposed to a series of nonsignal tones of constant or of variable intensity and performing tasks. Tasks include tests of attention using reaction time techniques, tests of perceptual speed using two-flash discrimination and tachistoscopic recognition, and tasks designed to be moderately stressful. A mini-computer system is used to run the experiments and to collect and analyze the data. Studies in various stages of completion are listed below.

1. Schizophrenia Studies

a. A study of newly admitted, drug-free patients used a "balloon stress" (blowing up a balloon until it pops) and two tests of perceptual speed given on different days. The tachistoscope task allows testing of the hypothesis, developed in previous studies, that schizophrenics' ANS does not respond appropriately to variations in stimulus significance. This study also includes several rest periods and a series of nonsignal tones for comparative purposes.

b. In current studies, ANS recording is being carried out in two sessions of rest, tone series, and reaction time. In addition, several methods of assessing attention deficits using reaction time (RT) techniques are being compared: (1) the classical "set" procedure of Shakow which involves variations in the foreperiod in a simple auditory RT paradigm, (2) RT to visual and auditory

stimuli, measured when the stimuli are predictable, unpredictable, or simultaneous (but unpredictably so). We have confirmed previous findings in normals that although RT to tones is faster than RT to lights when the stimuli are predictable, RT to light is faster under unpredictable simultaneous presentation. This is taken to indicate an attentional bias toward visual stimuli or visual dominance, (3) comparison of ipsimodal vs. crossmodal sequences of tones and lights in a simple RT paradigm, plus occasional simultaneous presentation to assess "intersensory facilitation." Simple RT is faster under simultaneous presentation of a tone and light in the context of an unpredictable series presumably because the subject's response is triggered by whichever of the two stimuli he is attending to.

c. Patients are being tested during their hospitalization using a protocol of rest periods, a series of variable intensity tones (60-100 dB), and a two-flash discrimination procedure. Patients in this study are on an active treatment or placebo. Drugs, such as pimozide, lithium, naltrexone, GHB, propranolol, and prazosin and other treatments, such as hemodialysis and plasmapheresis, are evaluated.

2. Studies on Nonschizophrenic Psychopathology

a. Patients with depressive and obsessive compulsive disorders have been tested shortly after hospital admission or as outpatients (on a protocol identical to the first part of the schizophrenics' protocol described in l.b. above) in collaboration with the CNB. Patients were tested while being treated with the tricyclic antidepressant clomipramine and the Type A monoamine oxidase inhibitor clorgyline as in l.c. above. Adolescent obsessive compulsive patients and aged-matched controls are also being studied in collaboration with LCS. (See Z01 MH 00336-05 CN and Z01 MH 00153-07 LCS.)

b. In collaboration with BPB, ten cases of multiple personality have been tested in 4-5 sessions each on short versions of the rest, tones, and RT time procedure. The method is to test the same three different personalities in a different order in each session to control for adaptation and compare the between-personality variance to the within-personality variance. Five normal controls have been tested in hypnotic or acting states. (See Z01 MH 00072-03BP.)

c. Men who had a diagnosis of early infantile autism are being tested with part of our standard protocol in collaboration with Drs. Rapoport and Rumsey. (See Z01 MH 00178-03 LCS.)

d. Patients with anxiety and panic disorders are being tested at baseline in collaboration with BPB. Further tests during drug protocols are planned. High ANS activity has been closely associated with anxiety disorders. This project may determine if variations in ANS activity are associated with clinical differentiations in this group of disorders and with response to different treatments. In addition this project is relevant to the questions of the specificity of ANS markers to particular disorders and the biological determinants of ANS activity. (See Z01 MH 00071-04BP.)

3. Studies on Normals

a. Two "high-risk" studies in which subjects were selected on the basis of performance on attention tasks, have been carried out. In one, subjects were selected for very good or very poor performance on the Continuous Performance Task, and in the other, pendulum eye-tracking was used. The procedures we have used were similar to those used in the current studies on schizophrenia.

b. Two studies of reactions to physical and psychological stress have been done in collaboration with LCS and BPB. In addition to ANS measures, measurement of changes in norepinephrine, beta-endorphin, opioid peptides and cortisol from plasma have been made.

c. In collaboration with LSES (Project #Z01 MH 00674 LSES), a method of confirmatory factor analysis is being used on ANS and personality data from 95 normal subjects. This has the objective of developing error-free measurement models of the structures of these systems, in order to reduce the large number of variables generated by the ANS and personality assessment procedures to many fewer and more "pure" concepts. This method may facilitate studying the interrelationships between systems.

4. Literature Review

A literature review of the psychophysiology of psychopathology was completed during the year. The review covers research using all psychophysiological techniques on the major psychoses, psychopathy, hyperactivity, autism, and neurotic disorders. This review should provide a framework for interpreting the data generated by this project.

C. Major Findings

1. Schizophrenic Studies

a. Analyses of the latest completed study with respect to the relationships of ANS activity to task performance diagnosis, symptoms, clinical course, biological markers, and the effects of treatment is continuing. The effects of prazosin, a specific alpha noradrenergic antagonist, in schizophrenics were an increase in ANS arousal but no clinical changes. The ANS effects are likely due to an increase in plasma NE. Since we have shown previously that schizophrenics as a group have elevated ANS arousal when drug free, clinical worsening might have been predicted from the ANS effects of prazosin. Instead, the results confirm our previous conclusion of an independence of variations in ANS activity and in psychosis which was based on data showing that only small decreases in arousal accompanied marked clinical improvement.

b. Data are still being collected. The new data continue to show that the phenomena of visual sensory dominance and intersensory facilitation found in normal subjects also occurs in schizophrenics.

2. Studies on nonschizophrenic psychopathology

a. Preliminary comparisons of obsessive compulsive adults with controls indicate higher baseline ANS arousal in the patients but no differences in habituation. This seems to be a distinct profile compared to other diagnostic groups, but definitive conclusions cannot be made without direct comparisons. In a group of adolescent obsessive compulsives, only the boys showed this pattern of results compared to matched controls. Obsessive girls had low arousal and ANS responsivity. Hopefully, correlations with other biological markers will shed light on this puzzling result.

Clinically, clomipramine was quite effective in reducing obsessional symptoms while clorgyline had minimal effects in most patients. Although the two drugs had rather similar effects in reducing electrodermal base levels, increasing HR and decreasing HR variability, compared to both placebo and clorgyline, clomipramine significantly reduced both tonic and phasic ANS reactions to simple tones (especially those of high intensity) and to the mild stress of the two-flash discrimination task. Subjects with a better clinical response tended to show greater drug effects on ANS reactivity. The results are compatible with the hypothesis that attenuation of ANS reactions to feared situations allows obsessive behavior to be extinguished.

b. Eight of nine multiple-personality patients had significant day-to-day consistency in between-personality variations in reaction time and in more of the ANS variables than would be expected by chance. The variables most likely to show such consistency were baseline arousal measures and ANS responses to simple tones and their habituation. Controls produced reliable consistency only through induction of hypnotic states, and it was less marked on the responsivity and habituation measures. Additional analyses revealed that in the multiple personality cases there was a partial carryover of habituation from one alternate personality to the next that was almost as large as that seen in the controls. This suggests an influence of the experience of one personality on the ANS reactions of others. The relationship of this to conscious awareness among alternate personalities will be investigated on a case-to-case basis.

c. & d. No reportable findings as yet as we are still testing patients and/or controls.

3. Studies on Normals

The major findings of these studies were described in last year's annual report. Papers on all of these studies are being prepared for publication.

Significance to Biomedical Research and the Program of the Institute

Investigations of ANS activity and attention in psychiatric disorders, especially schizophrenia, have produced promising results which suggest that these processes may play fundamental roles in the etiology and expression of the disorders. Limitations on inferences to be drawn from measures of ANS activity come from incomplete understanding of their biological and psychological

determinants. One of the main goals of this research is to increase this understanding by investigations of biological and psychological correlates and improving measurement techniques. The dynamic nature of these measures permits the study of processes, such as adaptation, habituation, response to and recovery from stress, and effects of single stimuli through noninvasive techniques. Thus, further understanding of their mechanisms could greatly increase their utility in investigations of psychopathology. Continued investigations of the diagnostic specificity of these processes and of their relationships to other clinical features and to prognosis are necessary to confirm and extend our previous results and to test the limits of their generality.

Proposed Course

Analysis of data will continue for the completed project on schizophrenia with the goals of determining the relationship of ANS variables to diagnosis, diagnostic subtype, symptomatology, severity of psychosis, performance on tests of attention and perceptual speed, degree of improvement during hospitalization and improvement on specific treatments. ANS activity in patient and control groups will be studied in relation to data obtained from biochemical assays of body fluids such as monoamines and their metabolites in CSF and monoamine oxidase activity.

Collection and analysis of data will continue for current projects on schizophrenic and nonschizophrenic psychopathology and in normal controls. This protocol will be used in the collaborative LPP project on attention disorders. Concept modeling by confirmatory factor analysis may be extended to other aspects of the large sample of controls and tried on data from schizophrenic patients. Investigation of ANS and behavioral effects of various pharmacological therapeutic agents will continue for all these groups with the purposes of determining the comparative effects of the drugs and correlates with clinical response.

We are planning to design a new protocol for schizophrenics by which to test the hypothesis that schizophrenics and controls exhibit differential effects of increases in arousal on attention. This will require choosing tasks that vary in their sensitivity to arousal manipulations. Arousal will be manipulated by changes in posture. Our experience with this technique in normal volunteers shows it to be effective in altering peripheral indicators of arousal without concomitant distraction.

Publications

Hommer, D.W., Zahn, T.P., Pickar, D., and van Kammen, D.P.: Prazosin, a specific alpha-noradrenergic receptor antagonist, has no effect on symptoms but increases autonomic arousal in schizophrenic patients. Psychiatry Res. 11:193-204, 1984.

Zahn, T.P.: Psychophysiological approaches to psychopathology. In Donchin, E., Porges, S.W., and Coles, M.G.H. (Eds.): Handbook of Psychophysiology, New York: Guilford Press, In Press.

Zahn, T.P., Insel, T.R., and Murphy, D.L.: Psychophysiologic changes during pharmacologic treatment of patients with obsessive compulsive disorder. British Journal of Psychiatry, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00486-12 LPP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Psychophysiological Effects of Stimulant Drugs in Children

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Theodore P. Zahn, Ph.D. Research Psychologist LPP, DCBR, IRP, NIMH
Other: Judith Rapoport, M.D. Chief SCP, LCS, NIMH

COOPERATING UNITS (if any)

Laboratory of Clinical Science, NIMH

LAB/BRANCH

Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION

NIMH/ADAMHA, Bethesda, Maryland 20205

TOTAL MAN-YEARS

0.3

PROFESSIONAL:

0.2

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Tests of the effects of acute and chronic administration of caffeine on autonomic nervous system (ANS) functioning have been carried out to evaluate the role of ANS activity in behavioral and subjective effects of this drug. A test of attention using a reaction time method is included.

The test protocol involves recording peripheral indicators of ANS activity such as skin conductance (SC), heart rate (HR), and skin temperature during a session consisting of a rest period, presentation of a series of simple tones to which no response is required, and the reaction time task. Studies have been carried out on the effects of the acute administration of two doses of caffeine and a placebo in 6-13 year old boys and in men, and a pilot study and major study of chronic (2 week) caffeine intake in children.

Results of the acute studies showed dose-dependent increases in such SC indices of arousal as frequency of spontaneous fluctuations and SC level along with retarded habituation of SC responses to the simple tones. HR showed a significant decrease after the low dose of caffeine and a partial return to the placebo level after the high dose. Results of our recent dose study on 41 boys and girls are similar for the SC variables, and there was a trend for decreased HR on caffeine.

The recent study confirmed the earlier ones in showing that while on placebo, subjects who were frequent caffeine consumers had lower SC arousal indices than non-consumers. These results, like those for caffeine effects, tended to be congruent with subjective reports of anxiety-like symptoms.

The SC effects of caffeine are much like those seen in patients with anxiety states and in normals after taking dextroamphetamine. However, HR results differ. These studies suggest that ANS activity and associated subjective states may play a role in the dietary choice of caffeine.

Project Description

This project has evolved from the study of hyperactivity in children (now called Attention Deficit Disorder) to the study of stimulant drugs-- dextroamphetamine and caffeine--in normal children and adults.

In previous studies, we found that frequent caffeine consumers had lower skin conductance arousal indices than non-consumers. The design of those studies did not permit separation of caffeine withdrawal effects from placebo effects. In the recent study, 41 children, selected for high or low caffeine use from a large sample, were tested while on their customary diet, after two weeks of no caffeine, and than twice more: after two weeks on either placebo or caffeine (5mg/kg bid) and then on the other condition. This design, of course, also allows assessment of the effects of caffeine taken in a more natural mode than in the acute studies.

In addition to the results reported in the Summary above, high and low consumers differed in their ANS response to caffeine. Low consumers showed a greater increment in SC level, but, surprisingly, high consumers showed a greater increase in spontaneous fluctuations. The latter result might be due, in part, to the high placebo levels on this variable shown by the low consumer group. Further analyses of the data are planned, including relationships of ANS changes to behavior and side-effect changes. (See #Z01 MH00161-06 LCS.)

The effects of chronic (two week) caffeine intake were similar to those seen after an acute dose, suggesting that tolerance does not develop, or that it is incomplete.

Significance to Biomedical Research and the Program of the Institute

The ANS effects of caffeine consistently found in these studies partially resemble those seen in anxiety states and other psychopathology. Caffeine has been shown to affect adenosine and benzodiazepine receptors. Thus it appears that ANS activity is partially controlled by these systems and may help mediate their effects on the symptoms of anxiety. These studies also suggest that dietary choices may be influenced by ANS activity. Finally, this demonstration of anxiety-like physiological effects of caffeine suggests that some persons may not tolerate it well. This would seem to have implications for the controversy over product labeling.

Proposed Course

Possibilities for future research in this area include psychophysiological study of children with anxiety disorders including a caffeine challenge. Another possibility is a detailed examination of the nature of the attention deficit in Attention Deficit Disorder in line with the general program of this laboratory to develop a taxonomy of attention disorders.

Publications

(See #Z01 MH 00161-06 LCS)

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00491-08 LPP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Personality Factors and Psychophysiological Responses to Changing Stimulus Input

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Theodore P. Zahn, Ph.D. Research Psychologist LPP, NIMH

Other: Thomas N. Robinson, Jr. Guest Worker LPP, NIMH

COOPERATING UNITS (if any)

NIH Normal Volunteer Office.

LAB/BRANCH

Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

0.5

PROFESSIONAL:

0.5

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The objectives of this project are to investigate relationships among differences in personality, sensory thresholds, and autonomic nervous system (ANS) activity in normal humans and to study racial differences in ANS activity. Bilateral skin conductance and heart rate have been recorded in two sessions in which constant and variable intensity tones and lights are presented and auditory and two flash thresholds (TFT) determined by methods which permit signal detection analyses. Several standardized personality tests were also given. These include scales of sensation seeking, extraversion, neuroticism, psychoticism, field dependence and anxiety. In addition comprehensive measures of lateral dominance have been given as well as a measure of "torque" (clockwise drawing of circles) which is thought to reflect a neurointegrative deficit and be related to risk for future psychopathology. The procedures allow determination of the effects of stimulus intensity and heteromodal stimulation on ANS activity. A procedure for manipulating ANS arousal experimentally with minimal distracting effects--a change in posture from supine to standing--is being used to assess the effects of arousal on performance and the effects of personality variables on this relationship. This project allows testing of several theoretical models of the relationships of ANS activity, sensory sensitivity, and personality, some of which have implications for the etiology of psychopathology. Tests of the relationships between laterality in skin conductance variables and behavioral laterality will also be done to see if inferences about lateralized brain function can be made from such variables.

Project Description

A. Objectives

A large body of psychological literature postulates that an important dimension of individual differences in behavior or personality is reflected in the reactions of the nervous system to sensory stimulation. Pavlov's original conception of "strong" and "weak" nervous types has been modified and extended by Western theorists to reflect such personality dimensions as "extraversion-introversion," "sensation-seeking," and "field dependence," each of which can be measured by a questionnaire or other test procedures. The theoretical models that have been built up from these concepts have implications for interrelationships among personality, autonomic nervous system (ANS) base levels and responsivity to stimulation, and sensory sensitivity. There are also implications for psychopathology in that schizophrenics have been considered to be extremely "weak" nervous types in the Pavlovian system (i.e., overreactive to weak stimulation and underreactive to strong stimulation--"transmarginal inhibition"). Another development is the more recent delineation by H. Eysenck of the dimension of "psychoticism."

The major objective of this project is to test some of the implications of these models of personality by interrelating the personality measures with sensory thresholds and sensitivity, and ANS activity in normal humans. Other objectives are to assess racial differences in ANS activity and in its relationships to the other variables in the study and to explore relationships of differences in the laterality of skin conductance activity with behavioral assessments of laterality, and to test the effects on ANS activity increasing arousal by means of a postural change.

B. Methods Employed

Over 200 normal volunteers have been assessed on several personality dimensions, including the Eysenck scale of extraversion, neuroticism, and psychoticism, field dependence, sensation-seeking, impulsivity, ego strength, and anxiety, assessed for degree of lateral dominance, and given tests of ANS and sensory functioning in two separate sessions as described earlier.

In a second protocol, the effects of changes in posture on ANS activity during rest, a series of 86dB tones and a TFT task is assessed. Subjects are tested when they are reclining or standing on two separate days in counterbalanced order.

C. Major Findings

Results from the first experimental protocol concerning the generally negative correlations of ANS activity with both the extraversion and, surprisingly, neuroticism dimensions were detailed in previous annual reports.

More recent analyses have concerned the Sensation Seeking (SS) scale of M. Zuckerman. These confirmed previous findings that men who score high on SS, particularly the disinhibition subscale, give larger ANS orienting reactions to a novel tone than low SS subjects. There was an interaction with state anxiety such

that the SS difference was large for subjects with high state anxiety. In general, state anxiety was inversely related to responsivity suggesting that the orienting response reflects alerting rather than emotionality.

The effect of a postural change from supine to standing was, as expected, to increase base levels of electrodermal and heart rate indices of arousal. In addition, the frequency and amplitude of skin conductance orienting responses to tones were also increased as were the amplitudes of the heart rate responses. Thus the postural change was successful in producing a change in ANS baseline arousal, and the change in responsivity was what might be expected from previous studies of between-subject correlations of baseline activity and responsivity.

Comparison of subjects along the personality dimension of "psychoticism" shows that those scoring high in this scale tended to have lower indices of ANS activity or arousal, particularly when reclining, than low scorers. High scorers also had poorer performance on the two-flash threshold task in terms of lower sensory sensitivity (d') and a less conservative criterion for detecting two flashes (β). The two-flash threshold was more subject to decrease with increases in arousal in the high psychoticism group as shown by both intragroup correlations of arousal indices and TFT and by the effects of standing. In general, the data show that high psychoticism scorers have unusually low arousal, sluggish orienting responses, and poor perceptual performance when reclining, and many of these variables tend to be "normalized" when standing.

Aside from the low sensitivity and criterion, which resemble findings from schizophrenics, the data for the high psychoticism group are more similar to those reported in the literature for subjects with primary psychopathy than for schizophrenics.

Subjects exhibiting a "torque" pattern of drawing a circle (clockwise motion), a pattern thought to reflect a neurointegrative deficit and increased risk for future psychosis, had impaired sensory sensitivity and low ANS baselines compared to nontorque subjects.

Significance to Biomedical Research and the Program of the Institute

Further understanding of how autonomic, perceptual, and personality variables interact in normal subjects should be of great assistance in interpreting the autonomic and perceptual results from studies on psychopathology in which similar methods are used in our other studies. Similarly, the study of racial differences in normals will help us evaluate the results of racially mixed samples of patients. This project has been very useful in the development of protocols for studies of psychopathology.

Proposed Course

Much further analysis of this large data base remains to be done, including assessment of laterality effects and determination of the effects of other personality dimensions in the most recent protocol. Techniques such as confirmatory factor analysis might be useful in such analyses.

We are planning also to develop a new battery of tests that vary in their sensitivity to arousal, making use of some of the recent developments in the field of cognitive psychology.

Publications

Robinson, T.N., Jr. and Zahn, T.P.: Psychoticism and arousal: Possible evidence for a linkage of P and psychopathy. Personality and Individual Differences, In press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00495-08 LPP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Psychobiology of Cognitive Processes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Herbert Weingartner, Ph.D. Chief, Unit on Cognitive Studies LPP NIMH

COOPERATING UNITS (if any)

Biological Psychiatry Branch, Laboratory of Clinical Science, Clinical Neuropharmacology Branch, Clinical Psychobiology Branch, NIMH; NIAAA; NIDA; Laboratory of Neurosciences, NIA (NIH); NINCDS (NIH); Walter Reed Hospital; USUSH

LAB/BRANCH

Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, Bethesda, Maryland 20205

TOTAL MAN-YEARS

2.0

PROFESSIONAL:

1.8

OTHER:

0.2

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- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The aim of these research efforts is to explore the psychobiology of cognition in man. We attempt to define the psychobiological determinants of the various components of cognition. Studies have been designed to understand the specific and discrete mechanisms which account for the acquisition, processing, encoding, consolidation, and retrieval of experience. Other studies have also begun to examine the meta-cognitive processes (self-monitoring operations) involved in learning and memory. Experiments are also designed to further our understanding of the biological and psychological determinants of impaired cognition in psychiatric and neuropsychiatric patients. Specific forms of central nervous system dysfunctions (e.g., as defined by type of lesion in neuropsychiatric disorders) may affect distinct components of cognitive processing. Similarly, psychoactive drugs that affect discrete aggregates of neurons, may alter different aspects of cognition and information processing and serve to model forms of cognitive dysfunctions in man. Based on empirical studies of clinical populations (e.g., depression, Alzheimer's disease, Huntington's disease, Korsakoff's disease, forms of learning impairments in children) and several types of psychoactive agents (cholinergic drugs, noradrenergic drugs, serotonergic drugs, drugs that alter GABA activity, neuropeptides), it has been possible to begin to define the psychobiological relationships between semantic and episodic memory, encoding processes, and effortful (active) cognitive operations as opposed to automatic cognitive processes.

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Project Description

The research projects reviewed here are all concerned with the psychobiology of cognitive processes. They have been designed to explore the psychological and biological determinants of various aspects of cognitive processes and their interrelationships. Studies reviewed here have examined and contrasted various types of cognitive disorders associated with psychiatric and neuropsychiatric diseases. Parallel research has examined how specific neurochemical systems mediate different types of cognitive processes. The types of processes that have been examined including the encoding, processing, learning, and storage of information, how processed events are altered, or elaborated in memory, the consolidation and retention of information, and the mechanisms that are involved in retrieval of stored information. Other research efforts have also begun to examine how we know what is and is not in memory (meta-cognitive processes), attentional determinants of information storage, aspects of short-term memory, the consolidation of information, long-term memory, state and trait specific cognitive strategies, effort demanding vs. automatic cognitive processes, and the kinds of strategies that subjects use to retrieve previously acquired knowledge and skills. Recent studies have also focused on the distinction between the psychological and biological determinants of episodic and semantic (knowledge) memory as well as meta-cognitive processes associated with information processing.

Two types of approaches or strategies are used to explore the psychobiology of cognitive processes. One is to contrast the effects of different treatments on different components of cognition. These studies include: (1) pharmacological manipulations, such as serotonergic drugs, drugs that alter GABA, cholinergic drugs, noradrenergic drugs, abused drugs (alcohol, marijuana), central nervous system depressants, neuropeptides, and (2) behavioral manipulations that alter reinforcing properties of stimuli, arousal/activation, stimulus attributes (altering encodability), and types of stimulus processing strategies subjects use to process information. These studies are carried out in unimpaired subjects, as well as in patient groups with different forms of psychopathology such as disorders of mood, dementing disorders and in patients with localized brain injuries. Would different kinds of pharmacological or behavioral manipulations of cognition lead to different forms of enhanced or disrupted cognition? Contrasting different treatment effects on different CNS systems and relating these changes to cognitive responses should be particularly useful in providing us with a picture of the structure of the psychobiology of cognition.

A second type of strategy for researching the psychobiology of cognition is to contrast different forms of cognitive failures as seen in different psychiatric and neurological syndromes. Would disruptions in cognition seen in some psychopathological states be qualitatively and quantitatively unique and related to specific changes in central nervous system activity or the neuropathology of these disorders? For example, what are the differences in the amnesic and cognitive impairments seen in Huntington's disease, Korsakoff's syndrome, and Alzheimer's disorder? To what extent are cognitive changes associated with aging or the "pseudodementia" evident in some Parkinson's disease patients different from impairments evident in dementing disorders? How might the differences be an expression of the specificity of central nervous system involvement in each of these disorders? In some instances, the possibility of discriminating between the form of the cognitive impairment is necessary for both adequate diagnosis and effective treatment, i.e., such as the cognitive disruptions that are part of depression as opposed to that seen in a progressive dementia of an Alzheimer's type. Frequently, depression is an integral part of a progressive dementia, and the cognitive impairment is a joint product of the two disorders. Some studies have also investigated the therapeutic potential of various psychoactive drugs and behavioral treatments. Do such treatments attenuate or reverse the cognitive disruptions seen in various forms of dementia, hyperactivity, amnesias, and learning disability syndromes in children, depression, and the schizophrenias?

In summary, each of the studies is clinically relevant but also serves as a basis for understanding the underlying psychobiological determinants of cognitive processes. Each project described below is concerned in some way with defining the discrete psychobiological components of cognitive processes that are involved in the appreciation, storage, retention, and retrieval of experience and using that knowledge to understand and treat disordered cognition.

1. Semantic (Knowledge) Memory and its Relationship to Other Forms of Learning and Memory (Episodic Memory)

Studies have been designed that would begin to describe how knowledge is represented in memory (semantic memory) and how it is accessed and used in order to encode or appreciate ongoing events. The relationship between semantic memory and episodic memory, how these are altered by various biological treatments and how these processes break down in neuropsychiatric disorders represents a very new and important area of investigation in exploring the psychobiology of cognition. It is also a particularly important area of study in order to understand the cognitive failures evident in such progressive dementias as in Alzheimer's disease.

2. Pharmacological Alterations (Enhancement and Disruption) of Cognitive Processes

Two types of studies have been completed. In one, drugs are used to simulate or model the varieties or types of cognitive dysfunctions that are seen clinically. Drugs such as scopolamine, ethanol, and benzodiazepine are used to develop such models. Other studies have contrasted the cognitive enhancing effects of drugs which differ in terms of their effects on central nervous system activity. Neuropharmacological strategies that have been of particular interest include serotonergic agents (Zimelidine); dopaminergic drugs (L-dopa/carbidopa); cholinergic agents (such as arecoline, physostigmine, THA, and lecithin treatment); amphetamine; and various forms of drugs that alter neuropeptide activity (vasopressin, naloxone). These drugs have been administered to both unimpaired subjects, as well as various cognitively impaired patient groups (hyperactive children; patients suffering from various forms of dementia such as Alzheimer's and Huntington's disease, Korsakoff's disease, aging and depressed patients). Do drug treatments that affect different aggregates of neurons in the central nervous system produce different kinds of changes in cognitive processing? Are enhancements or disruptions of cognition determined through different psychobiological mechanisms, and might the cognitive response to different drugs make such a pattern discernible? Might some neurotransmitter antagonists also mimic or model forms of syndrome specific disorders of cognition in man? In some instances, drugs that might disrupt aspects of the acquisition of information may enhance some other stage of cognitive processing (e.g., consolidation). Neuropharmacological tools that, on the one hand, model forms of disturbed cognition, or strategies that prove useful in treating cognitive dysfunctions can also be used to better define a psychobiology of information processing in man. This research has also involved attempts to find clinically useful drug treatment strategies for altering disrupted cognition in man.

3. State-Dependent Learning

This area of research has provided a useful framework for exploring: a) the qualitatively unique manner in which events are stored (encoded and later retrieved from memory); b) how multiple personality configurations can serve as context markers in the encoding and retrieval of information; c) studies of disturbances in mood state and how these define mood-specific strategies for

processing experience and remembering past events in memory; d) qualitative changes in cognition in response to psychoactive drugs; e) contextual factors as determinants for defining the nature of trace events in memory; and f) individual differences in susceptibility to state-dependent or dissociative mood/drug effects.

4. Memory Consolidation

This research has focused on the psychobiological events that follow the acquisition (storage) of information and the events that occur before processed information is to be retrieved from memory. Studies in both normal subjects and patients have examined the form and strength of stored information in memory and the processes that might further sustain, enhance, or disrupt stored trace events that are already part of memory. Information in memory is altered in time, including changed in form. The experience of knowing that some event occurred earlier also changes as memories are transformed during a consolidation phase of information processing. The psychobiological events that play some role in the rate of decay of information in memory and the susceptibility of information to interference may be important determinants in defining what is available and accessible in recall, once information has been stored in memory. Disruptions in consolidation may contribute to the cognitive failures in the dementias as well as in amnesic syndromes.

Drugs that disrupt memory and learning may do so by altering biological operations that succeed acquisition or learning. Likewise, drugs (e.g., neuropeptides) may enhance aspects of learning and memory by facilitating the consolidation of learned information.

5. Behaviorally-Defined Mechanisms that Alter Components of Cognition

A number of behaviorally defined processes have been explored in forms of how these alter cognitive performance. These include characteristics of stimuli such as: a) organizational and relational properties; b) imagery inducing properties; c) emotional arousing attributes of stimuli; d) information presentation rate; e) mode of processing; f) acquisition of language vs. pattern information; g) kinds of learning such as automatic vs. effort demanding cognitive operations; and h) priming of information in knowledge memory. These factors have each been studied in relation to its effects on attention, acquisition (learning), strength of learning, retention, and components of information retrieval. The studies have examined these factors in normal controls, as well as in patients groups, (depressed patients, hyperactive children, learning disabled children and patients suffering from various forms of dementia). Some of the issues raised in these studies include the following: How might aspects of information processing alter the attentional, short-term memory, encodability, retention, and retrieval of information? Would different forms of psychological manipulations systematically alter different aspects or components of cognitive processes? Do patients who demonstrate failures in learning and remembering do so because of disruptions in some, but not all of these component cognitive processes, and can manipulations of some characteristics of information processing change or attenuate these disruptions in cognition?

Recent studies have also begun to explore the relationship between the reward-reinforcement systems along with "effortful" vs. "automatic" information processing and how these are altered under different motivation/arousal conditions. In addition, new studies have explored ways of altering rapid access to knowledge and making knowledge available in encoding new events, thereby making them more memorable. This approach to cognition has been particularly useful in defining the cognitive changes in depression, the determinants of cognitive failures in the learning-disabled child, pseudementia in contrast to cognitive impairments in progressive dementias of an Alzheimer's type and drug-altered changes in cognition (see below).

6. Cognition and Mood

Studies have included research of mood-related changes in: a) depression in relation to brain lateralization of cognitive functions; b) arousal and activation and its role in information processing; and c) the encoding and retrieval of events in unimpaired subjects where mood also can be a variable as well as in disordered mood in depression and mania. This research has examined how patients with disturbances in mood process information in a mood-state specific manner. In addition, this research has begun to examine mood-related changes following psychoactive drug treatment and its interactive role in altering cognitive processes. Other research has explored the degree to which effortful processing of information is compromised as a motivation-related determinant of thinking in depressed patients.

7. Mechanisms of Cognitive Impairments that Determine Forms of Learning Disabilities

Studies have been designed which investigate: a) forms and incidence of various kinds of learning disabilities in children; b) the nature of the learning disabilities in these children; and c) potential strategies for their remediation.

Methods

Three strategies have been used in these studies. One involves manipulation of different biological systems that may play a role in different aspects of cognition in man. Various neurotransmitter agonists and antagonists, as well as agents that affect neuroendocrine functioning are contrasted in both impaired and unimpaired subjects. A second strategy involves systematic comparison of various forms of cognitive failures apparent in different clinical groups. Methods used include measures and assays for evaluating neuropathological, neurochemical, and neuroanatomical changes that are apparent in different clinical syndromes. These data provide a matrix for relating biological variables with measures of different components of cognition as seen in forms of impaired cognition. The third set of methods involves systematic manipulation of acquisition conditions, stimuli, retention processing, and retrieval conditions.

Several types of cognitive procedures and strategies have been designed to explore the psychobiological components of cognition in the studies that are

part of this project. Many of these methods are modified forms of current techniques used in human information processing research, as well as newly-developed tools that might more adequately examine determinants of cognition in clinical studies and those assessing cognitive drug effects. These strategies have been developed in a number of studies and include measures and manipulations of: a) organization of information; b) informational context for processing information; c) stimulus attributes such as imagery, emotional properties and frequency; d) forms of learning and recall (free recall, prompted free recall, recognition memory, cued recall, serial learning and paired associates learning); e) processing time and type of presentation of information; f) type of processing strategy (processing on the basis of meaning or sound properties); g) immediate vs. delayed recall of information with or without rehearsal of stored information; h) presentation of language vs. pattern information to left vs. right hemisphere (methods used to investigate lateralization); i) rapid (tachistoscopic) presentation of information; j) measurement and manipulation of different forms of retrieval of information in memory, including forms of free recall, prompted or cued recall, recognition memory, method of "savings"; k) very long-term memory retrieval; l) assessment of effortful and automatic cognitive processes; and m) arousal and motivation in information processing.

Most recently new cognitive methods have been developed which also permit us to examine characteristics of semantic (knowledge) memory in contrast to the methods described above which are primarily useful for describing episodic memory. These methods allow us to measure the structure of knowledge in memory and how readily it can be accessed and used in transforming events. This is being accomplished through the development of new behavioral techniques as well as psychophysiological and neurobiological methods (positron emission and versions of event related and average evoked response methods).

Findings: Psychobiology of Cognition

1. Semantic Memory, Episodic Memory, Automatic and Effortful Cognitive Processes: Psychobiological Relationships

A series of studies has shown that semantic memory (knowledge memory) and episodic memory are psychobiologically distinct but interactive cognitive systems. Studies have shown that a) failure to access semantic or knowledge prostructures in memory is the major determinant of the cognitive failures in many progressive dementia patients of an Alzheimer's type (SDAT); b) that episodic memory failures are determined by semantic memory impairments in this type of progressive dementia; c) other amnesic syndromes (such as in Korsakoff's disease) are determined through different psychobiological mechanisms, other than those that affect access to semantic memory; and d) the cognitive disturbance in depression is due to a specific disruption in effort demanding cognitive operations while automatic processes and semantic memory functions are left relatively unaffected - this same pattern of cognitive impairment is also evident in early stage Parkinson's disease. These findings have been useful not only because they elucidate basic mechanisms of learning, memory and related cognitive cesses but they provide new and more effective diagnostic tools for distinguishing types of cognitive dysfunctions.

Related neuropharmacological findings include: a) neuropeptide treatments such as arginine vasopressin facilitate access to semantic memory; b) serotonergic drugs, such as Zimelidine appear to enhance memory by amplifying weak, poorly processed memory traces; c) L-dopa treatment appears to produce a specific enhancement of effort-demanding cognitive operations implicating the dopamine system in this type of memory-learning function.

Behavioral manipulations that induce priming of knowledge in long-term memory has a direct and positive effect on episodic memory performance. This new finding is also being exploited in the development of pharmacological strategies that would aid memory-impaired patients.

2. Cognitive Impairment in Progressive Dementia, "Pseudodementia" and Korsakoff's Disease and Possible Treatment Strategies

Recent findings from our laboratory have defined some of the characteristics and determinants of the cognitive dysfunction in progressive dementia patients. We know that information is relatively rapidly lost from memory; immediate memory is relatively unimpaired, and any type of learning-memory operation that requires the establishment of permanent trace events in memory is dramatically disrupted. Memory failures are, in large part, due to processing or acquisition deficits which then result in weak trace formation and therefore failures to retain information in memory. A considerable body of research has suggested a distinction between semantic memory and the repository of information of knowledge structures from episodic memory, i.e., memory for ongoing recent events. Although these two kinds of memory systems have been traditionally viewed as being separate and distinct, we have found an important link between the two. Based on recent findings relating these two systems, it has been possible to account for many aspects of the memory impairment in progressive dementia patients. In a series of studies, we have been able to demonstrate that the extent to which Alzheimer's patients have access to structures in semantic memory is the extent to which they are relatively unimpaired on many tasks of learning and memory. These results have important implications both diagnostically, in distinguishing this group of cognitively impaired patients from other groups (e.g., cognitively impaired depressed patients), as well as for the development of potential treatment strategies.

Parkinson's Disease (PD) patients also demonstrate learning-memory problems that can be quite severe. We have found these impairments to be qualitatively different from those evident in Alzheimer's disease. PD patients manifest impaired cognition on effort demanding cognitive tasks but not when information can be processed relatively automatically. Access to semantic memory is also left unaffected in the early and middle stages of the disorder. L-dopa treatment, a common drug used in PD, produces a facilitation of these same cognitive component processes in unimpaired older subjects.

Most recently we have been able to show some facilitation of learning and memory in progressive dementia patients using two very different strategies. Cholinergic drugs seem to produce small improvements in learning and memory but only in those patients that are least cognitively impaired. In contrast, arginine vasopressin enhances learning and memory by facilitating access to

semantic memory (a mechanism of action that is consistent with the determinants of the memory failure in these patients).

Although Korsakoff patients (KD) are often as memory impaired as progressive dementia patients, the cognitive and biological determinants of their impairments are quite different. Unlike progressive dementia patients, the Korsakoff amnesia patient responds to attributes of stimuli that would ordinarily aid encoding such as a) repeating information, b) organizing information, and c) presenting pictures rather than words. Furthermore, the Korsakoff patient (KD) can learn procedures and remember them for very long periods of time. This is because unlike progressive dementia patients the Korsakoff patient (KD) is able to access semantic memory.

In attempting to reverse the amnesic-like impairment in KD we have tried drugs that would affect the noradrenergic system. Thus far we have been unsuccessful in producing reliable improvements in KD memory functions using clonidine as a drug strategy.

These findings, when examined together, have suggested that cognitive failures in progressive dementia are distinguishable from those evident in depression and other syndromes. This has prompted active study of drug and other treatment strategies for reversing such cognitive failures. By understanding both the mechanisms of cognitive impairments and the neurochemical response following various forms of drug treatment, it should be possible to design studies that would examine the therapeutic potential of various types of drug treatments. The mechanisms and determinants of the cognitive impairments in depression and dementia have allowed us to devise strategies that should prove useful in distinguishing between these two groups of patients. Characteristics of automatic versus effortful processing, the extent to which effort is extended in accomplishing tasks, and the processing of unrelated vs. related events allows us to begin to differentially diagnose the cognitive impairment in depression from that seen in the progressive idiopathic dementia patients.

Newly developed methods will allow us to identify cognitively impaired patients who are more likely to benefit from different types of drug treatment. Other recent research has provided new approaches to behavioral rehabilitative techniques as well as approaches to longer term drug treatment in cognitively impaired patients.

3. Cognitive Changes in Depression

The pattern and determinants of cognitive changes in depression have been shown to be distinguishable from those expressed in other disorders (particularly in early stage progressive dementia). Depressed patients demonstrate a type of disordered thinking, one that is manifest in an inability to accomplish focused, sustained analysis of information leading to impairments in concept learning, acquisition of information, and memory. This may be related to alterations in the function of cerebral lateralization involved in processing language vs. non-language information.

A series of studies have been designed to examine the effects of focal, CT scan defined, lateralized brain lesions on mood (depression) and cognition. As expected, non-dominant hemisphere lesions produce cognitive dysfunctions that are systematically different from those produced by dominant hemisphere lesions. Non-dominant hemisphere lesions of the temporal-parietal region are also associated with profound states of depression that are obvious to trained observers but are not experienced, as such, subjectively. In contrast, patients with dominant hemisphere lesions experience depression that is consistent with observer (clinically evaluated) rated depression: in addition, the severity of cognitive dysfunction is highly correlated with the severity of depression in dominant hemisphere patients.

4. Learning Disabilities in Children

Drug treatments, such as stimulants, appear to facilitate learning and memory in some types of learning disabled children. These cognitive effects are seen primarily for those processes that require sustained effort. These effects are apparent and independent of other clinical changes in these amphetamine-treated children. In addition, learning that occurs in the amphetamine-treated state does not appear dissociated when remembering takes place in the untreated state. This is not like the kinds of dissociative, state-dependent, learning and memory effects that are seen in stimulant treated adults. These results are also important in considering the effects of stimulant treatment on the educational experience of learning disabled or hyperactive children.

In a series of studies, we have attempted to describe the components of cognitive changes that are apparent in children with various forms of learning disability. We have examined two groups of these children, one where hyperactivity is part of the syndrome, and a second group where there is no evidence of hyperactivity or generalized retardation. Nevertheless, these children demonstrate dramatic impairments in learning and memory that resemble the kinds of disruptions in cognition that are evident in some groups of adults. The resemblance is closest to depressed patients; it also resembles the kinds of cognitive changes that are produced by drugs that disrupt cholinergic and noradrenergic activity. These children show impairments in effortful processing of information; automatic processing is left relatively intact. On incidental learning paradigms, these children are indistinguishable from normal controls. Both groups of children also demonstrate impairments in those characteristics of cognition that require the imposition of organization in memory. In many ways, the results we have obtained to date would suggest that the type of cognitive impairment seen in these children resembles that seen in depressed patients in contrast to the pattern of cognitive impairments evident in progressive dementia patients. The kinds of cognitive impairments are also like those that are apparent when unimpaired subjects are treated with drugs that disrupt or block catecholamine activity.

5. Meta-cognitive Processes in Amnesic Syndromes and Schizophrenia

Recently completed studies have demonstrated that unimpaired subjects are sensitive to many aspects of their own processing operations and memory for previously acquired events. Unimpaired subjects "know" how well something has

been learned and the likelihood that something remembered is likely to have occurred or to have been part of the memory reconstruction processes involved in remembering events. Seriously impaired progressive dementia patients, despite their memory failures, can accurately judge whether an event was part of their reconstruction of their memory for previously processed events as compared to recall of some trace of that event. Korsakoff psychosis patients, who are similarly memory impaired, cannot accurately judge characteristics of their own memory. They demonstrate what might be conceptualized as a dissociation or delinking between limbic (old brain) systems and the processing associated with neo-cortical areas. In recent experiments we have also noted this same type of cognitive dysfunction in schizophrenia patients.

6. Neuropharmacological Studies of Cognition in Man

We have been able to demonstrate, both in patient groups as well as in unimpaired subjects, that the effects of cholinergic antagonists and agonists produce cognitive changes that are qualitatively different from those of drugs that have their major effect on catecholamine activity. There appears to be further specificity and distinctiveness in the role of neuropeptides such as synthetic vasopressin-like substances, and of naloxone, in determining aspects of learning and memory in both cognitively impaired patients (depressed patients, alcoholic Korsakoff amnesic syndrome patients and progressive dementia patients) as well as in unimpaired subjects. Different neurotransmitter systems and different kinds of neurochemical mediators are involved in the regulation of various aspects of episodic memory (acquisition, retention, and retrieval of information) while other biological determinants appear to influence semantic memory processes. Furthermore, effortful cognitive operations appear to be determined by different biological mechanisms from those involved in automatic cognitive operations.

We have demonstrated that cholinergic mechanisms play a role in aspects of information acquisition and in the storage and retrieval of information. Scopolamine treatment, which disrupts cholinergic activity, produces an impairment in information processing. This scopolamine-induced impairment in the acquisition of new learning can be reversed by arecoline treatment. The scopolamine induced disruption in cognition appears to model, in normal subjects, many of the characteristics of cognition seen in untreated progressive dementia patients.

In another study, it was possible to show that cholinergic mechanisms may also be involved in the consolidation of information in memory. When subjects learn information in a drug free state, and are treated afterwards with arecoline, there is a facilitation of information later recalled.

Amphetamine treatment also increases the amount of information which can be recalled following various modes of input processing under drug state conditions. Unlike cholinergic manipulations, amphetamine appears to amplify or strengthen trace events in memory rather than increasing the total amount of learning (size of the pool of trace events in memory). In a series of studies, it has been possible to show that amphetamine produces an enhancement of some components of cognition (in depressed patients, hyperactive children, normal

children, and normal adults). Amphetamine also induces a change in state which serves as a state-specific context biasing how information is interpreted and remembered. Amphetamine treatment, like cholinergic treatment, produces state-dependent retrieval. The contrasting enhancing effects of cholinergic agents and amphetamine and the cognitive disrupting effects produced by scopolamine vs. lithium have served as one strategy for exploring the specific psychobiological mechanisms that may define different components of cognitive processes.

While alcohol has been viewed as one type of pharmacological manipulation that reliably produces learning and memory impairments in man, recent work from our laboratory in collaboration with NIAAA has demonstrated that post-processing manipulations (including treatment with alcohol) can in fact produce some enhancements in learning and memory. The focus on the biological and psychological events that follow the initial acquisition of information has generally been ignored in studies of cognitive processes. This consolidation phase of memory and the biological events that occur during this time may be important in establishing permanent records of experience. This is evident both in our studies using alcohol, where we saw evidence for a paradoxical enhancement of what has been stored in memory, and in the effects of vasopressin on reversing retrograde amnesia following ECT administration. We noted that alcohol, when administered after the processing of information, produces an enhancement in recall when tested in the unintoxicated state. This has been interpreted as an effect on memory consolidation. Other related findings suggest that alcohol induces a brief excitatory phase (possibly mediated by changes in catecholamine activity) which affects memory consolidation. This excitatory phase may be important in defining some of the reinforcement properties of alcohol. This paradoxical cognitive facilitating effect of alcohol, administered during a consolidation phase of memory, appears to highlight the differentiated mechanisms and components that make up information processing, memory, learning, and retrieval.

We have now completed a series of cholinergic trials in Alzheimer's patients and have demonstrated that cholinergic antagonists such as scopolamine mimic many of the characteristics that are evident in progressive idiopathic dementia. We have also noted that combinations of cholinergic agonists do in fact produce small but reliable enhancements of some aspects of learning and memory in patients with Alzheimer's disease. The limiting factor here has been that the extent to which an enhancement in learning and memory is evident is largely a function of the degree to which cognitive functions are preserved in these patients.

Some of our most recent studies have also demonstrated the specific role of the dopamine system in modulating effort demanding cognitive processes but not information processing that can be accomplished automatically. We have demonstrated that when older normal volunteers are treated with L-dopa (in combination with carbidopa) memory for effortfully processed events is enhanced while memory for automatically processed information is unaltered. This finding has important implications for our basic scientific understanding of the psychobiological processes that determine cognition. At the same time these findings can be translated into appropriate clinical applications in the treatment of cognitive improvements.

In another set of studies we have also begun to explore the role of the serotonin system in learning and memory. We have shown that the drug Zimelidine (a relatively specific 5 HT reuptake blocker) can substantially reverse the commonly seen cognitive impairing effects of ethanol. This finding is also important because of its basic scientific implications as well as its clinical value in the treatment of alcohol related cognitive impairments.

In a series of studies it has been possible to begin to accurately model different types of cognitive dysfunctions associated with neuropsychiatric syndromes. Benzodiazepine produces an anterograde learning-memory impairment that is quite similar to that seen in Korsakoff's Disease. Attention and episodic memory are disrupted in a dose-dependent fashion while access to knowledge memory is essentially spared. In sharp contrast, scopolamine disrupts access to knowledge memory, thereby, affecting other aspects of cognition. This pattern of cognitive changes models that seen in senile dementia of an Alzheimer's type.

This type of drug modeling of cognitive impairments has provided us with new leads and approaches for the development of cognitive remediation strategies as well as a powerful framework for better understanding the psychobiology of cognitive dysfunction.

Summary

The recently completed programmatic-research efforts of the Unit on Cognitive Studies has extended our knowledge of the psychobiological structure and determinants of cognition. Completed research has been valuable in better defining disordered mood, the nature of the information processing impairments in progressive dementia, Korsakoff's disease, and other alcohol-related disorders, and the nature of cognitive defects in learning disabled children. Neuropharmacological studies in both unimpaired subjects and patient groups have provided us with further information about the neurochemical events important for learning, memory, and cognition. These studies have also provided new approaches in the treatment of various forms of cognitive disturbances.

Specifically, we have begun to describe the major psychobiological differences and relationships between recent (episodic) memory and knowledge (semantic memory). This distinction between these two types of memory systems is important for our understanding of forms of cognitive failure in man. We have developed a way of characterizing the nature of the cognitive changes in depression and Parkinson's Disease, and used this as a way of examining the psychobiological distinction between automatic and effortful episodic memory-learning processes. It has been possible to model forms of cognitive impairments in man such as those that are seen in Alzheimer's disease, in drug studies of unimpaired subjects (cholinergic antagonists). This type of research has helped us in our efforts to facilitate aspects of cognition in these patients using cholinergic agonists. Neuropeptides have also been used to treat some of these cognitive disorders (e.g., arginine vasopressin). We have also modeled disorders of information processing in unimpaired subjects (with the use of naloxone). Based on our recent studies that involve changes in the dopamine and serotonin system we are also able, for the first time, to differentially

affect automatic vs. effortful cognitive operations and to alter recall of weak memory traces in contrast to well learned information in memory. As a result of these research efforts it seems important that we focus our new efforts in the following areas: (a) mediational processes that are involved in transforming and encoding information (using psychophysiological and neuropharmacological tools); (b) the relationship between the reward system and memory processes, particularly as these would alter memory consolidation; and (c) new ways of facilitating impaired cognitive processes.

Significance to Biomedical Research and to the Program of the Institute

These research efforts have a direct bearing on how diagnoses of cognitive dysfunction are accomplished and the directions of future efforts for treating the cognitive impairment associated with a wide variety of psychiatric and neuropsychiatric disorders.

Proposed Course

We hope that current studies will lead to better diagnostic tools and effective therapies for cognitive dysfunction.

Publications

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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00500-05 LPP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cognitive and Perceptual Changes in Affective Illness

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Edward K. Silberman, M.D., Guest Worker, LPP/NIMH

Others: Dr. Robert Post, Biological Psychiatry Branch, NIMH
 Jean-Phillippe Boulanger, Biological Psychiatry Branch, NIMH
 Linda Bierer, Biological Psychiatry Branch, NIMH
 Thomas Uhde, Biological Psychiatry Branch, NIMH
 Dr. Rex Cowdry, Clinical Director, DCBR, NIMH
 Sander Genser, M.D., Walter Reed Army Institute of Research

COOPERATING UNITS (if any)

Biological Psychiatry Branch
 Walter Reed Army Institute of Research

LAB/BRANCH

Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

3.0

PROFESSIONAL:

2.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is to investigate the cognitive and perceptual changes which are present in, and characteristic of major affective illness and its various clinical and biological subtypes. Two separate studies make up the overall investigation: (1) psychomotor and psychosensory symptoms in patients with affective illness, (2) lateralized hemispheric function in depression.

Project Description

This project is based on the idea that cognitive and perceptual changes in depression may be useful markers for sub-classification of affective disorders. At present, there are two distinct approaches to investigating such markers, which are described below.

1. Psychomotor and Psychosensory Symptoms in Affective Illness

In our initial study we have compared 44 patients with major depressive disorder to 37 patients with partial complex epilepsy and 30 hypertensive controls on the frequency with which they experience transient alterations in perception, ideation, affect, and motor behavior, similar to symptoms which have been described as seizure concomitants in the clinical epilepsy literature. In our initial study, both affective and epileptic patients reported such symptoms with significantly greater frequency than controls. Both affective and epileptic patients reported transient visual, auditory, and olfactory changes. Epileptic, but not affective patients reported gustatory, vestibular, and tactile phenomena, as well as involuntary motor symptoms. Affective patients were distinguished by presence of cognitive illusions, and distortions of time perception. Such symptoms were experienced by affectively ill patients primarily during episodes of illness rather than interval periods. High frequency of symptoms report was unrelated to personality factors as measured by MMPI. With the exception of an inverse relationship to age, symptoms were not related to demographic variables, or parameters reflecting course of illness. However, those patients with better response to lithium and tricyclic antidepressants reported higher numbers of symptoms than those with poor response to these drugs.

Proposed Course

At present the focus of planned research is to investigate psychosensory symptoms in patients with other diagnoses in the affective spectrum and to study the relationship of such symptoms to clinical presentation of depressive illness, to biological parameters, and to drug response. In conjunction with Dr. Robert Post, Jean-Phillippe Boulanger, Linda Bierer, and Thomas Uhde of the Biological Psychiatry Branch, NIMH, we have investigated the occurrence of psychosensory symptoms in patients with panic-anxiety disorders, with and without concomitant depression. Plans are under way to examine the relationship of these epileptic-like symptoms to antidepressant response to carbamazepine, a drug useful in treating both affective disorders and epilepsy.

2. Lateralized Hemispheric Function in Depression

Many lines of evidence suggest that two cerebral hemispheres differ in the manner and degree to which they process emotionally-related stimuli and modulate emotional behavior. In particular, the right hemisphere has been found to exhibit "dominance" for processing of emotions, overall, or as processing of negative emotions specifically. In affective disorder, where disturbances of mood and emotions are paramount, a variety of studies have suggested that the right hemisphere is disordered in its function, or that the balance of activity

level between right and left hemispheres is shifted in favor of the right. An initial investigation from our laboratory demonstrated that, in hospitalized, depressed women, the right hemisphere appears to more efficiently process verbal stimuli which are normally handled by the left hemisphere. This finding supports the hypothesis that the right hemisphere is in some sense "hyperactive" in depressive illness.

Proposed Course

Two follow-up studies are being planned. In the first, a group of patients with bipolar illness who cycle in and out of depressed and elevated mood states will be studied. The measure of lateralization will be based on the procedures in the initial study; subjects will be asked to make decisions about verbal material presented tachistoscopically to the right and left visual fields. Proportion of correct responses and reaction times will be used to infer the site and efficiency of processing of the verbal task. The study will be designed to (a) replicate the finding of hemispheric shifts in depression, (b) investigate hemisphericity in pathologically elevated moods, and (c) distinguish between such shifts as state related vs. traits of patients prone to affective illness. We will use a repeated measures design in which patients serve as their own controls, and the main within-groups factor is mood state.

In the second study, we will use our procedures to examine laterality in patients with a significantly depressed mood (as measured by standard clinical scales) in the context of a wide variety of disorders within the affective, schizophrenic, and personality areas. The aim of such a survey would be to learn to what extent laterality changes are related to depressed mood itself, to diagnostic categories (e.g., affective illness, but not personality disorder with depression) or to subtypes within affective disorders. Laterality may also be an indicator of prognosis, degree of recovery, or response to medication, and such parameters will be investigated. These studies will be conducted in collaboration with Sander Genser, M.D., Walter Reed Army Institute of Research. The patient population for the studies will be drawn from the inpatient services of the Walter Reed Army Medical Center.

Significance to Biomedical Research and to the Program of the Institute

These investigations are a part of a basic research at NIMH aimed at elucidating the nature of affective illness. Cognitively related studies are relevant to this goal from three points of view: (1) they concern an important area of deficit in affective illness, (2) they define an aspect of dysfunction which may provide clues to the pathologic anatomy and physiology of affective illness, and (3) they may provide useful information relating to clinically meaningful classification of affective disorders.

Publications

Silberman, E.K., Post, R.M., Nurnberger, J., Theodore, W., and Boulanger, J.P.: Transient sensory, cognitive, and affective phenomena in affective illness. A comparison with complex partial epilepsy. Br J Psychiatry, in press.

Silberman, E.K., Weingartner, H., Stillman, R., Chen, Hong-Jen, and Post, R.M.:
Altered lateralization of cognitive processes in female depressed patients. Am.
J. Psychiatry. 140: 1340-1344, 1983.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00503-04 LPP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Human Clinical Studies of Attention Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Allan F. Mirsky, Chief, LPP, NIMH

COOPERATING UNITS (if any)

Epilepsy Branch, Clinical Neurosciences Branch, NINCDS; Laboratory of Clinical Science, NIMH; Boston University

LAB/BRANCH

Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA Bethesda, Maryland 20205

TOTAL MAN-YEARS:

3.0

PROFESSIONAL:

1.75

OTHER:

1.25

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This research comprises three related areas of investigation concerned with specifying neuropsychological factors underlying clinical conditions in humans in which disturbed attention is a major symptom. A major emphasis is on (1) illuminating the nature of brain stem pathophysiology, if any, in such entities as petit mal or absence epilepsy, infantile autism, schizophrenia, and related diseases; (2) an additional major emphasis is on extending the neuro-behavioral analysis of attention loss in absence epilepsy so as to facilitate developing alternative treatment strategies for such patients. Both of these projects form part of a larger effort which is aimed at (3) developing a comprehensive and systematic taxonomy of attentional disorders in humans. This latter study will eventually comprise study of patients with cerebral lesions, seizures, dementing diseases, and metabolic illnesses of the brain.

Other Professional Personnel

Connie C. Duncan-Johnson, Ph.D., Chief, Unit on Psychophysiology, LPP, NIMH
 Richard Coppola, D.Sc., Senior Engineer Officer, LPP, NIMH
 Herbert Weingartner, Ph.D., Chief, Unit on Cognitive Studies, LPP, NIMH
 Theodore P. Zahn, Ph.D., Research Psychologist, LPP, NIMH
 Richard Nakamura, Ph.D., Senior Staff Fellow, LPP, NIMH
 Roger Porter, M.D., Chief, EBB, NINCDs
 Judy Rumsey, Ph.D., Staff Fellow, LCS, NIMH
 Debbi Fein, Ph.D., Asst. Professor of Psychiatry (Neuropsychology), Boston Univ.
 John M. Morihisa, M.D., SMRA, NIMH
 Daniel R. Weinberger, M.D., SMRA, NIMH

Project Description1. Brain Stem Mechanisms in Attention Impairment

Current approaches to the neuropsychology of attention impairment have emphasized that the system responsible for the maintenance of attention or consciousness within the brain is most likely represented at a variety of levels of the neuraxis. From an evolutionary point of view, it is clear that the capacity for sustained attentive behavior is present in many species which do not possess more than a rudimentary forebrain or telencephalon. MacLean's analysis of the R-complex within the human brain leads to the view that this "clump of ganglia," which constitutes virtually all of the reptilian brain, can support a variety of ritualistic, repetitive behaviors which could be characterized as sustained and attentive. Evolution progressed and the brain developed additional complexity and volume. Additional capacity for attentive behavior was thus overlaid on the more primitive, although in many aspects thoroughly adequate, brain stem system of the reptile. Therefore, although the system for maintenance of attentive behavior in the human (or higher primate) includes limbic and neocortical components, the brain stem remains a key component and possibly the keystone of the entire system. Authors such as Hughlings Jackson and Penfield and Jasper recognized this in their conceptions, respectively, of "highest level seizures" and the "centrencephalon." In their theorizing, consciousness was either localized in or regulated by deep brain stem structures. Without reviewing all of the evidence that led to those views of the hierarchical organization of attention and consciousness within the brain, we nevertheless point to the extremely deleterious effects on such capacities of small lesions in the brain stem region of the third and fourth ventricles. In the last ten years, a new technological refinement of evoked potential methodology has made possible an other-than-theoretical exploration of the role of brain stem structures in certain clinical states. This "far field" technique makes it possible to assess the integrity of auditory (and somatosensory) relay nuclei within the brain stem of humans. Although the technique has probably had most utilization in the diagnosis of demyelinating disease, it has also been used in the study of other neurological and, recently, psychiatric disorders. There may or may not be any specific interest in these sensory systems (auditory, somatosensory) in studying a particular clinical entity (i.e., absence seizures, infantile autism);

nevertheless, the possibility of evaluating the functional integrity of certain systems within the brain stem is extraordinarily valuable, and many clinical investigators are using these techniques. We have published work indicating that there are disturbances (prolonged transmission time) in the processing of auditory information in the brain stem in infantile autism. We have also shown that in absence seizures (spike-wave activity), both naturally-occurring and experimentally-induced, there may be perturbations of auditory brain stem functioning. We have run approximately 13 autistic children and a smaller number of controls on brain stem evoked potentials. The results to date indicate considerable disorganization in the potentials of the autistic subjects.

2. Neurobehavioral Studies in Absence Epilepsy

We have for a number of years been studying the absence attack in patients with petit mal/centrencephalic/absence seizures (the terms are more or less interchangeable) as a model state to understand the phenomenon of consciousness/attention. Some of these studies have involved comparing the behavioral capacities of patients suffering from petit mal--as opposed to focal seizure disorders; other studies have involved detailed comparison and contrast between the behavioral and the electroencephalographic symptoms/signs of the disorder. Most recently these investigations have: (1) used evoked potentials in the visual and auditory modalities as indices of the sensory effects of generalized seizure activity of the symmetrical and synchronous spike and wave (SW) variety, and (2) examined changes in the EEG power spectrum prior to SW bursts as prodromal signs which may be used to predict (and ultimately to control) SW bursts. We propose to continue this line of neurobehavioral investigation, using event related potentials of various types as well as other behavioral and physiological tools, to refine further our understanding of the nature of altered consciousness in absence (petit mal) epilepsy.

3. A Taxonomy of Attentional Disorders

The goal of this project is to develop a comprehensive and coherent account of the relation between symptoms of altered or disturbed attention or consciousness as they appear in various clinical entities, the other behavioral and clinical characteristics of the several disorders, and the specific central nervous system damage or disturbance in each disorder. The attentive capacities of the patients will be assessed by a number of measures comprised within the GAT (generalized attention test) which is an outgrowth of the CPT (continuous performance test) a measure of sustained visual attentive behavior. The ultimate goal will describe the precise attentive deficit (as opposed to cognitive losses) and the nature of the neuropathophysiology associated with each of the following clinical entities:

- cerebral cortical lesions (frontal, parietal, or temporal lobe)
- centrencephalic/absence epilepsy
- schizophrenia
- infantile autism
- dementing diseases (Alzheimer's, Korsakoff's, Huntington's).
- metabolic diseases (Phenylketonuria, Uremia, Anorexia Nervosa and related illness)

We will attempt, as well, to relate these changes where possible to standardized measures of mnemonic and other cognitive function, and to autonomic indices of attention, arousal, and habituation.

The EEG imaging laboratory has been carrying out a variety of mapping studies over the past year. We now have the capability of following the dynamically changing aspects of EEG and ERPs in real time allowing direct visual observation of the spatial distribution motion of brain activity patterns as they change in time.

(a) Trial of intravenous procaine hydrochloride: 24 subjects have now been tested and the first statistical analysis completed. Significant changes in EEG patterns have been formed: an increase in frontal delta, decrease of alpha, and increase of fast activity that localizes to temporal regions as frequency increases. These changes are consistent with the hypothesis that procaine causes limbic system activation. As our N increases we hope to dissect this result by clinical group.

(b) Patients with Alzheimer's disease: We have now run more than 20 patients with an additional 20 well relatives or spouses. Preliminary analysis has indicated specifically, abnormal, alpha distribution maps at other frequency bands for the sick individuals compared to controls.

(c) Seizure patients: We now have EEG maps on more than 30 patients with a variety of seizure disorders. With the real time imaging system it has been possible to study the dynamics of the EEG both in terms of amplitude maps during epileptic events and for frequency maps before and during seizure. Several subjects with complex parietal epilepsy have shown focal areas of fast activity interictally for EEG records that would have been read as normal. It appears that analysis and imaging may be able to reveal EEG abnormalities, who otherwise would have gone unnoticed. We have two absence patients from whom numerous seizures were recorded. We are preparing an analysis to determine if there are any discriminative features in the EEG just prior to seizure.

(d) Studies of psychoactive compounds and schizophrenia: Eight subjects have now been run from 4E, on and off drug, and we are preparing a preliminary analysis.

An EEG imaging laboratory has been set up at St. Elizabeth's in collaboration with Dr. Morihisa. Three clinical studies are under way: Schizophrenia, Alzheimer's and EEG images. In collaboration with Dr. Weinberger we have already produced color activity maps from CBF data. The simultaneous recording will allow direct correlation between blood flow and EEG images in a more rigorous way than in previous studies.

Significance to Biomedical Research and to the Program of the Institute

Since attention disturbance is a characteristic of many significant psychoneurological disorders, it is essential to have a clear empirical and theoretical account of the role and pathophysiological significance of the symptom. It will aid in understanding the etiology and course of these illnesses:

and may aid in improving their treatment.

Proposed Course

We have run a small group of schizophrenic, epileptic, and brain-injured patients through our laboratory procedures (i.e., CPT, brain stem auditory evoked potentials, various tests of cognition and memory, autonomic indices of attention, etc.). During the course of the next year, we hope to recruit additional cases from other diagnostic categories into this taxonomic study. However, since we have not had a laboratory to pursue this work, it has not been possible to achieve substantial progress during this reporting period.

Publications

Browne, T.R. and Mirsky, A.F.: Absence (Petit mal) Seizures. In Browne, T.R. and Feldman, R.G. (Eds.): Epilepsy-Diagnosis and Management. Boston, Little Brown, 1983, pp. 61-74.

Coppola, R.: Psychophysiological monitoring of lifestyle. Psychologia. 26: 190-194, 1983.

Kaye, W.H., Ebert, M.H., Gross, H. and Lake, C.R.: Catecholamine metabolism in anorexia nervosa. In Lake, C.R. and Ziegler, M. (Eds.): The Catecholamines in Psychiatric and Neurologic Disorders. in press, 1984.

Kaye, W.H., Ebert, M.H., and Gwirtsman, H.E.: Brain serotonergic metabolism differentiates between patients with anorexia nervosa that fast or binge. Am J Psychiatry, in press, 1984.

Kaye, W.H., Ebert, M., Jimerson, D., and Lake, C.R.: Enduring abnormalities in norepinephrine metabolism in anorexia nervosa, 5th. International Catecholamine Symposium, Goteborg, Sweden, 1983. In abstracts, Supplement to Progress in Neuro-Psychopharmacology and Biological Psychiatry. New York, Pergamon Press, 1983, pp. 186.

Sitaram, N., Weingartner, H., Kaye, W.H., Ebert, M.H., and Epstein, R.: Combination Treatment of Alzheimer's Dementia. In Reisberg, B. (Ed.): Alzheimer's Disease, The Standard Reference. Macmillan, Inc., 1983, pp. 355-361.

Proposed Course

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Coppola, R.: Psychophysiological monitoring of lifestyle. Psychologia. 26: 190-194, 1983.

Kaye, W.H., Ebert, M.H., Gross, H. and Lake, C.R.: Catecholamine metabolism in anorexia nervosa. In Lake, C.R. and Ziegler, M. (Eds.): The Catecholamines in Psychiatric and Neurologic Disorders. In Press, 1984.

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Kaye, W.H., Ebert, M., Jimerson, D., and Lake, C.R.: Enduring abnormalities in norepinephrine metabolism in anorexia nervosa, 5th. International Catecholamine Symposium, Goteborg, Sweden, 1983. In abstracts, Supplement to Progress in Neuro-Psychopharmacology and Biological Psychiatry. New York, Pergamon Press, 1983, pp. 186.

Sitaram, N., Weingartner, H., Kaye, W.H., Ebert, M.H., and Epstein, R.: Combination Treatment of Alzheimer's Dementia. In Reisberg, B. (Ed.): Alzheimer's Disease, The Standard Reference. Macmillan, Inc., 1983, pp. 355-361

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00504-04 LPP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Models in the Monkey of Generalized Seizures of the Absence Type

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Allan F. Mirsky, Chief, LPP, NIMH
 Others: Eva Bakay Pragay, Ph.D., Research Psychologist, LPP, NIMH
 Michael Myslobodsky, M.D., Ph.D., Professor, University of
 Tel Aviv, Israel

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

0.7

PROFESSIONAL:

0.7

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Generalized seizure activity with the electrographic appearance of absence epilepsy (bilaterally symmetrical and synchronous paroxysmal three-per-second spike and wave discharges) can be elicited in the monkey by a variety of methods. These include electrical stimulation of various locations within the brain, injection of convulsant drugs and other substances, and administration of compounds which may alter normal inhibitory mechanisms within the cell. Model seizure states created in these ways are studied in order to test hypotheses about pathophysiological seizure mechanisms, sensory processing and attentional capacities during absence seizures, effects of spike-wave activity on cellular activity, and effects of techniques or maneuvers which may modify or reduce convulsive activity. Most recently this project has involved the following work: we studied the (paradoxical) seizure inducing effects of a GABA-enhancer and the effects on auditory brain stem evoked potentials of generalized seizures induced by injection of pentylenetetrazol.

Project Description

γ -vinyl GABA and γ -acetylenic GABA are two recently synthesized compounds whose metabolic effects include the blocking of the enzyme action responsible for the metabolism of the inhibitory neuro-transmitter GABA. The accumulation of GABA thus produced should have an anticonvulsant action, and so it does, at moderate doses of these compounds. However, as the dose is increased, there is a paradoxical rebound effect and animals treated with large quantities of either γ -vinyl or γ -acetylenic GABA have shown paroxysmal seizure activity. And of interest to us is the fact that the seizure activity is not the clinically obvious generalized tonic-clonic variety. Instead, although widespread spikes and spike-wave patterns may be seen, there may be few clinical signs. Such an effect is reminiscent of absence seizures (staring spells) in human centrencephalic epilepsy. We are in the process of exploring the utility of these compounds for producing model seizures of the petit mal variety in the monkey.

We have also induced generalized seizure activity in rhesus monkeys, reflected in both clinical and EEG manifestations, by systemic administration of pentylenetetrazol. Brain stem auditory evoked potentials (BAEP) were recorded from indwelling epidural electrodes at the vertex of the skull as well as from electrodes implanted along the primary auditory pathway in the brain stem (inferior colliculus). Several components of the complex "far field" vertex potential showed increased latency and decreased amplitude during ictal episodes as compared to control periods both pre-drug and post-seizure. Similar changes were seen in direct recordings from brain stem auditory structures. The parallel recording of "far field" (vertex) potentials and "near field" (brain stem auditory pathway) potentials appears to be a fruitful approach. The direct recording from brain stem auditory structures adds reliability and temporal resolution to the findings. Thus, in contrast to the vertex potential which requires several hundreds, or even thousands of stimulus repetitions, only a few samples are necessary to obtain reliable waveforms from direct brain stem recordings. Consequently, the grain and the resolution of the experiment can be enhanced, and various periods of pre-, during- and post-ictal stages as well as phases of gradual recovery can be assessed. The analysis of small consecutive samples revealed profound BAEP changes not only during the ictal period but immediately following the seizure activity. There was also marked fluctuation of suppression and potentiation during the post-ictal recovery period.

Significance to Biomedical Research and to the Program of the Institute

This experiment provides direct evidence of brain stem involvement in consciousness and in generalized seizures and contributes to the current efforts to produce an accurate primate-based model of the pathophysiological processes in absence epilepsy.

Proposed Course

We will be continuing with this experimental program as primate facilities become available to LPP. Although we have had no opportunity to pursue animal model studies in this project, there has been progress this year of a scholarly nature. Together with various intramural and extramural scientists, plans are

being developed to publish a book reviewing the recent developments in the biochemistry, electrophysiology and genetics of absence epilepsy. The book will incorporate much of the material germane to this project. Furthermore Dr. Michael Myslobodsky is contributing to a review article on absence epilepsy being prepared jointly with members of the LPP.

Publications

None

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00505-04 LPP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Brain Lesion and State Change Effects on Visual Processing and Attention

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Richard K. Nakamura, Ph.D., Senior Staff Fellow, LPP/NIMH

Others: Mortimer Mishkin, M.D., LN/NIMH

Richard Wyatt, M.D., William Freed, Ph.D. APB/NIMH

Louis Skoloff, M.D., Charles Kennedy, M.D., LCM/NIMH

Carolyne Smith, Ph.D. LCM/NIMH

COOPERATING UNITS (if any)

Laboratory of Neuropsychology, Laboratory of Cerebral Metabolism, and
Adult Psychiatry Branch, NIMH

LAB/BRANCH

Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

3.0

PROFESSIONAL:

2.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project consists of four related areas of investigation, all concerned with the analysis of mechanisms of information processing and attention. Special emphasis is placed on the use of lesions, physiological recording, and metabolic mapping techniques to elucidate mechanisms involved in visual attention. The four areas are: (1) attention and cerebral mechanisms of visual behavior; (2) pharmacological mechanisms of attention; (3) brain activity in inattention and sleep, as measured by glucose metabolism and protein synthesis; and (4) physiological studies of selective visual attention.

1. Attention Mechanisms and Visual Behavior

We have found that large nonvisual lesions of cerebral cortex in the monkey cause permanent blindness. The basic preparation is as follows: one hemisphere is visually deafferented by combined optic tract transection and forebrain commissurotomy. The other hemisphere has all cortical areas removed except for the striate, prestriate and inferior temporal visual areas. Animals prepared in this way were functionally blind for over a year despite anatomical evidence of an intact visual system. We have been analyzing this phenomenon in an effort to determine the role of nonvisual cortex in visual behavior and to establish the nature of higher processing in the visual system. The major results indicate that we are beginning to elucidate the role of attention in sensory processing.

We have examined the single neuron responses to visual stimuli in the visual systems of our blind monkeys. Results of over 400 neurons studied to date in the blind animals compared to over 200 neurons in normal animals indicate that visual responses of the blind are near normal in both striate and prestriate areas. Preliminary data from inferior temporal cortex, on the other hand, suggest that the blind animals show less specificity of neuronal response than seen in the normals. These data imply that in the absence of feedback from higher cortical structures and in the absence of behavioral feedback, the visual system continues near normal processing through to the prestriate cortex. Only at the level of inferior temporal cortex does the effect of such feedback losses change cellular responses to visual stimulation. This in turn suggests that the effects of attention on neuronal responses will not be significant in areas earlier in the visual pathway than inferior temporal cortex.

In an effort to determine the critical cortical zones of this blindness phenomenon, the large nonvisual ablation has been subdivided into three parts: the sensorimotor cortex, the limbic cortex, and the polysensory cortex. These areas have been ablated in separate groups of monkeys and the only lesion which produces a blindness effect is the polysensory cortical lesion. This area consists of dorsal prefrontal cortex, inferior parietal cortex, and superior temporal cortex (including insula). It is of considerable significance to us that a neglect or inattention syndrome follows the ablation of any portion of the polysensory area for this suggests that our animals cannot see because they cannot attend to the visual modality.

The absence of effect of the sensorimotor lesion creates confidence that the blindness is not simply the result of a disconnection of visual input from motor output. In the original chronic blindness preparation, the non-visual lesion is placed in one hemisphere, the optic tract to the other is cut and the forebrain commissures are divided. If, however, the forebrain commissures are left intact, then there will be a period of blindness lasting approximately 10 to 40 days. This period is followed by a recovery of visual function which permits not only visual guidance to food, but discrimination of visual patterns as well.

This recovery of visual function, when a path of communication is left between the hemispheres indicates that the blindness is the result of a disconnection of vision from higher cortical processing areas. Combined with earlier results, we can be more specific and say that the blindness is caused by

a disconnection of visual areas from polysensory (attentional) areas of the brain. Thus a connection between visual and attentional areas appears to be critical before visual behavior can proceed in monkeys.

2. Pharmacologic Mechanisms of Attention

Striatal dopamine has been implicated as an important transmitter in the attention-arousal system. Areas A9 and A10 (substantia nigra pars compacta and the ventral tegmental area) have been shown to be major sources of dopamine for the brain in general and the cerebral cortex in particular. In rats, lesions of areas A9 and A10 have been associated with inattention or neglect, tremor, transient aphasia and adypsia, and a deficit on the delayed alternation task. Little is known about the effects of such ablations in the monkey though in man cell loss and reduced dopamine in these areas have been associated with Parkinsons disease.

Because of our interest in striatal dopamine, we have begun a study with the Division of Special Mental Health Research, Adult Psychiatry Branch, to investigate the possibility of transplanting dopamine secreting tissue into the brains of monkeys that have been previously deprived of striatal dopamine with unilateral 6-hydroxydopamine ablations. We have found that autografts of adrenal medulla can be successfully implanted into the caudate nucleus of monkeys with substantia nigra lesions. These grafts survive up to one year after implantation and appear to produce dopamine. Unfortunately, in contrast to experience with the rat, relatively few cells survive in the monkeys tested and fewer still develop a neuronal phenotype. We are currently trying different approaches to increase the yield. It is possible that transplantations in primates present more difficult problems than in rodents.

3. Brain Activity in a State of Inattention

Major clues to the systems involved in attention might be derived from the study of natural states of reduced attention such as sleep. In collaboration with the Laboratory of Cerebral Metabolism, the Laboratory of Neuropsychology, and the Sleep Laboratory, we have been studying cerebral glucose metabolism of monkeys in slow wave sleep and wakefulness. A total of eight monkeys has been examined, four experimental animals in slow wave sleep and four control animals that were kept awake. The major finding has been that the animals in sleep show an overall reduction in cerebral metabolism of about 30%. Further, we have been unable to find any brain structure which shows, on average, higher activity in slow wave sleep than in the awake state. Hypnogenic center theories, which postulate a brain area which actively keeps an animal in sleep, are therefore not supported. The loss in attention which accompanies slow wave sleep thus appears to be the result of a general loss of brain activity.

In addition, we have applied a new method to determine local cerebral protein incorporation in another eight monkeys, four in slow wave sleep and four that were awake. Data from these animals suggests that protein synthesis, unlike cerebral glucose metabolism, is increased throughout the brain in slow wave sleep. Theories of sleep suggesting that protein synthesis increases during sleep to make up for deficits incurred during wakefulness are therefore

supported. This is a particularly exciting result since it establishes a strong reason for the need to sleep, something that has eluded us for centuries.

4. Physiological Mechanisms Underlying Information Processing and Visual Attention

We are attempting to map information processing across the cerebral cortex of the monkey based on cortical evoked responses. Previous analyses have suffered from lack of sensitivity, reliability, replicability, or interpretability. Such problems are reduced in this investigation by a variety of strategies, the most important being: a) arrays of bipolar transcortical (surface to depth) electrodes, b) a highly controlled behavioral task with several variations, and c) a large number of trials (2000) per day.

Electrodes are placed over the cortical surface of one hemisphere with sulcal impressions in the concavity of the skull as landmarks. The basic task is a go/no-go visual pattern discrimination that is reversed daily. The visual stimuli are presented for 100 ms on a shutter controlled screen. Task variations include: changing visual patterns, manipulating brightness, using an auditory stimulus, requiring all-go responses, and altering the percentage of correct go responses that are rewarded. Evoked responses recorded from electrodes in an animal performing this task are band passed at .1 to 100 Hz and sampled at 200 times/sec.

In the three monkeys tested thus far we found high reliability over time in signals from individual electrodes. Evoked response patterns were also replicated in both form and response to task manipulation at corresponding brain sites across animals. Responses at individual sites were sensitive to different aspects of the task including: stimulus pattern, stimulus meaning, stimulus modality, motor response, and reward delivery. Responses measured at different sites showed considerable variation even when they were only a few millimeters apart. In general, electrodes in the occipital and inferior temporal cortex were sensitive only to visual information whereas parietal electrodes anterior to the visual system were sensitive mainly to the motor response or reward delivery. Evoked responses in the precentral gyrus reflected not only the motor response but stimulus and reward delivery as well. Prefrontal electrodes showed the greatest variety of responses.

The power of this approach lies in its potential to summarize information processing across all the cytoarchitectonic regions of the cortical mantle simultaneously. Our results to date suggest that we now have the necessary sensitivity, reliability, and replicability to fulfill this potential. Below are described two of the findings that have been generated by this project.

A. ERP analysis of events underlying reaction time differences in the monkey.

Reaction time (RT) is a ubiquitous physiological and behavioral measure that must often be treated as univariate. The spatial and temporal sensitivity of our system for mapping cortical information processing in the monkey allows differentiation of the cerebral events underlying an animal's RT. This is clearly illustrated by a double dissociation of effects following two different manipulations that reduce RT.

First, RT can be reduced by increasing the brightness of a visual trigger stimulus. However, the relative contributions of the various CNS processing stages to the overall changes in RT with brightness have not yet been firmly established. On our go/no-go visual pattern discrimination task, an 8:1 brightness increase in the visual patterns produced a savings of approximately 30 ms in an overall RT that averaged approximately 300 ms. An analysis of the evoked cortical responses indicated that the earliest clear peaks, which appeared at 89 ms to the dimmer stimuli, occurred 24 ms earlier to the brighter stimuli. This suggests that 80% of the RT difference is attributable to precortical delays which occur in the first quarter of the total RT. Since peaks appearing after 100 ms show the full RT difference, the entire RT savings is accounted for by processing that is completed in the first third of the total RT.

Second, RT can be reduced by simplification of the decisions required before a response. We compared reaction times when an animal is required to do the standard go/no-go task to those when the animal has to 'go' on all trials. This task yielded a 20 ms savings in RT. The latencies to all the identifiable evoked response peaks prior to the motor peak (as seen in the precentral gyrus recording) were within 3 ms of each other. On the other hand, the motor peak reflected the full 20 ms difference. The last identifiable peak before the motor peak is at 180 ms. Thus all the RT savings in this case occurred in the last third of total processing time. In contrast to the case of brightness manipulation where larger stimulus-related peaks were associated with faster reaction times, in this case, smaller stimulus related peaks were associated with faster reaction times.

B. ERP analysis of visual input to precentral gyrus (motor cortex).

While studies have shown that the precentral gyrus contains neurons that respond to visual and auditory stimuli, there is little agreement about the significance of these responses. We have implanted arrays of transcortical electrodes in monkeys trained to do a go/no-go visual discrimination task. Evoked responses from electrodes in the precentral gyrus of the monkey showed a characteristic set of waves. The earliest component, which begins at approximately 65 ms, was tightly time-locked to the onset of the stimulus and appeared in exactly the same form and amplitude whether or not the animal made a 'go' response. This component first appeared 5-10 ms before any detectable activity in visual cortex and had a polarity opposite to that seen in the visual cortex. A later component predicted and was time locked to the response of the animal. This started by 150 ms and, on go trials, peaked just after the response which occurred between 250 and 330 ms. Although the sensory component of the evoked response appeared widely in the precentral gyrus, it was very weak or nonexistent in both postcentral gyrus and prefrontal cortex.

Varying the brightness of the stimulus affected both the amplitude and latency of the sensory component in the precentral gyrus as well as in the visual cortex. Unlike visual cortex, however, the precentral gyrus was not sensitive to changes in the stimulus pattern. Further, manipulation of task parameters that change the meaning of the stimulus could also influence activity in visual areas without doing so in precentral gyrus. The differences between

the patterns of evoked responses in visual cortex and precentral gyrus of latency, form, and response to manipulations of meaning raise the possibility that the signal in precentral gyrus was derived from a noncortical visual area such as the optic tectum.

One monkey that has been trained to respond to auditory stimuli showed a sensory component in the precentral gyrus similar to the one evoked by visual stimuli but at a 20 ms latency. This appeared only after the animal had been trained to respond quickly to the auditory signal.

These results suggest that the precentral gyrus receives behaviorally relevant sensory information and may derive this information from a subcortical source. This function of the sensory input may be to prepare the motor system for a fast stimulus-triggered response.

Significance to Biomedical Research and to the Program of the Institute

We hope to understand the mechanisms underlying attention and consciousness in animals and man. This will enable us to develop adequate animal models of human clinical syndromes featuring reduction of attention or consciousness, such as schizophrenia, dementia, and petit mal epilepsy.

Proposed Course

Projects 1,2,and 3 are nearly complete and are gradually being terminated. Papers are in progress for all of them. Project 4 is rapidly being expanded because of its great promise.

Publications

None

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00506-04 LPP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Attention-Related Neurons in the Brain of the Rhesus Monkey

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Eva Bakay Pragay, Ph.D., Research Psychologist LPP/NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, Bethesda, Maryland 20205

TOTAL MAN-YEARS

3.2

PROFESSIONAL:

2.2

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project is concerned with an analysis of the activity of nerve cells in that system within the primate brain which is necessary and responsible for the process we refer to as attention. Monkeys trained to perform visually-guided go, no-go discrimination tasks are tested whilst extra cellular recordings are made from brain regions thought to be part of an attentional system. The most recent study in this series examined structures in the forebrain.

The exploration of the frontal cortex now has been completed. We found attention related units in the prefrontal cortex including the prearcuate, the periprincipalis and anterior cingulate areas. These cells responded to the manipulation of attention, e.g., manipulation of the pre-stimulus waiting period or changing the behavioral significance of the task-stimuli. These attention-related units have properties similar to those found in the brainstem reticular formation in previous studies.

Project Description

The present study represents a further step in our long-standing goal to identify brain structures which support attention functions. In this project we record unit activity in the macaque brain in conjunction with the administration of a successive visual discrimination (attention) task. In essence this is a mapping study which involves application of consistent experimental conditions while exploring the cellular activity in various target as well as control areas. The target areas which are thought to be involved in attentional functions include the "non-specific" brainstem areas as well as cortical associational areas. The specific motor and visual areas serve as control areas. In earlier studies we explored the brainstem reticular formation (RF) and the prefrontal and anterior cingulate areas as assumed parts of an attention-system, while the premotor, motor and posterior cingulate regions served as controls. In the past year we reassessed the prefrontal target areas and extended our exploration to the most anterior aspects of these regions.

In all these studies, the task required the animal to press a "hold" button for 2 seconds in order to turn on a "cue" button; the latter was transilluminated by either a red ("go") or a green ("no go") cue-light. In the go trials, the animal had to release the hold button and press the cue button within 1 second. In the no go trials, it had to maintain pressure on the hold button for another second. In the basic task, both correct go and no go trials were rewarded.

The task permitted us to distinguish response-related (Type I) and stimulus-related (Type II) cell types. Type I units, which responded only during go trials, can be regarded as related to the execution of the instrumental motor response. Type II neurons which respond during both go and no go trials were considered to be attention-related if they responded to the behaviorally important aspects of the stimulus, and if they responded to the manipulation of attention. Such manipulations included varying the length of the intertrial interval or changing the behavioral importance of the stimulus by withholding the reinforcement which follows the correct response to the stimulus in the standard task.

Units with such characteristics were found in all the target areas explored so far (that is, the brainstem RF, the prearcuate and anterior cingulate areas). To our surprise, a small number of units located in the control areas explored so far (that is, the premotor-motor cortex and the posterior cingulate region) also showed some of "attentional" characteristics. In other words, target and control areas showed a certain degree of similarity despite marked differences. These findings could be best described in terms of an anterior-posterior gradient rather than in terms of a target-control contrast. According to this gradient the attention-related characteristics diminished gradually in the frontal cortical area as one moved from the arcuate sulcus to the central sulcus.

The most recent data obtained from more anterior aspects of the dorsolateral and dorsomedial prefrontal cortex supported our prediction in that there were marked differences from the posterior aspects of the frontal lobes. The difference was manifest not only in the higher proportion of Type II (stimulus-related) units in the anterior vs. posterior regions, but also in marked

morphological and apparent functional differences across Type I and Type II subtypes. Type I units in the prefrontal region showed a relatively weak and often irregular response; also, their increased activity started after the behavioral response. This was not true for the majority of the posterior Type I units. More important is the marked A-P differences seen in Type II subtypes. While the majority of the posterior Type II units were "asymmetric" in that the go response had increased firing frequency and duration as compared with no go trials, the majority of the anterior Type II units were "symmetrical", in terms of similar responses in both kind of trials. In addition, we found some anterior Type II units which responded to both the onset and the offset of the stimulus, and some of them were also anticipatory to both stimulus events. In our standard task the duration of the no go trial (and the presentation of the no go stimulus) was longer than the go trials. Therefore "on-off" units were morphologically asymmetrical in terms of longer lasting activity on the no go trials, although the total magnitude of their go and no go response was similar. Consequently, these units could be rendered more symmetrical by equalizing the duration of two kinds of trials.

Some of the characteristics described above reflect functional significance. Thus, those Type I units in the anterior regions which begin their increased activity after the onset of the behavioral response cannot be involved in the mobilization of that response, although they may serve some feedback functions related to the occurrence of the motor response. In contrast, the vigorous and relatively early onset of the posterior Type I units most likely indicates involvement in the mobilization and or execution of the motor response. This assumption is supported by the fact that the activity of these posterior units was closely correlated with the onset of the motor response.

In the Type II subtypes, the units with symmetrical and/or anticipatory activity may have general activating-facilitatory functions. Their temporal qualities in terms of earlier onset in the anterior vs. posterior regions may indicate that the anterior frontal areas may lead in the activation process. The facilitatory character of the anticipatory activity is underlined by the fact that the anticipatory increase brought about by increasing of the intertrial interval resulted in decreasing of the behavioral reaction time.

The asymmetric posterior Type II units may be described in functional terms as visuo-kinetic. Their visual characteristics can be best assessed by their no go response; their dual involvement is represented by their increased response in the go trials. The motor involvement is also underlined by the fact that their go response is better aligned to the onset of the motor act rather than to the onset of the stimulus. In addition, the unit activity in the erroneous no go trials ("commission errors") was similar to the activity in the go trials. These units have all the properties to serve as links between stimulus and response. As such, they may be involved in the visual triggering of the motor response, and/or in the visual guidance in the execution of the motor response. The distinction between the posterior and anterior forms of a symmetry may also be stated in functional terms. While the stimulus-related activity in the posterior units does not reflect any visual property of the stimulus, the anterior on-off units reflect one visual aspects, i.e., duration. However, the responsiveness to this stimulus property is not a passive reflection, for two reasons: 1) some of these on-off

units anticipate these stimulus events, 2) these stimulus events at the same time are signalling a behaviorally important event seems to be a prefrontal characteristic. One sample contains units which are more active around the end of the trial and others which are excited only at the end of the trial. Some of these units may be influenced by manipulating reinforcement. These units show marked changes in terms of decreased activity in conjunction with the absent reinforcement. Some other late units do not respond to the manipulation of reward, and others show increased "excitement" in conjunction with the missing reward, both in the case of erroneous trials or in conjunction with the experimental withholding of reinforcement in correct trials. Thus the cellular reaction to the missing reinforcement is another issue which illustrates anterior-posterior differences.

Significance to Biomedical Research and to the Program of the Institute

Our study is relevant to three major issues: the localization of attention; the functional organization of the prefrontal cortex; the functional relation of prefrontal cortex to other cortical areas of the forebrain (with special emphasis on the premotor, supplementary motor and motor areas). The study of attention is important because attention deficit is a characteristic symptom in many psychopathological and neurotic conditions.

Proposed Course

The exploration of cortical associational areas for attention-related units will be extended to the posterior parietal cortex. In addition to the single unit activity, we plan to record multiple unit activity and gross evoked potentials from the same area. This study will be carried out in collaboration with Dr. Richard Nakamura.

Publications

Mirsky, A.F. and Bakay Pragay, E.: Brain mechanisms in the processing of sensory information: Clinical symptoms, animal models, and unit analysis. In D.E. Sheer (Ed.): Attention: Theory, brain functions, and clinical application. Hillsdale, N.J., Erlbaum, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00507-02 LPP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical Brain Imaging

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Robert M. Cohen, M.D., Ph.D., Acting Chief, CBI, LPP, NIMH

COOPERATING UNITS (if any)

Neuroscience Branch, NIMH; Biological Psychiatry Branch, NIMH; Dept. of Psychiatry, University of California at Irvine; Clinical Center, NIH; Nuclear Medicine, CC, NIH; Adult Psychiatry Branch, NIMH at St. Elizabeths Hosp.

LAB/BRANCH

Laboratory of Psychology and Psychopathology

SECTION

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INSTITUTE AND LOCATION

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TOTAL MAN-YEARS

6.5

PROFESSIONAL:

3.2

OTHER:

3.3

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Investigators in this section are continuing to refine existing methodologies for the study of cortical functioning on the basis of positron emission tomography (PET) and electrical brain mapping procedures in humans.

As wide variability has been noted in normals and patient groups in terms of PET determinations of cerebral glucography, efforts have been made to control for this. First, we have attempted to control behavioral variability through having subjects participate in specific psychological tasks during these studies. This has included the use of a somatosensory stimulus paradigm and most recently an auditory continuous performance task (cpt). Secondly, we have attempted to use methods of statistical analysis that deemphasize absolute glucose metabolic rate in favor of comparative regional rates.

Using these approaches, schizophrenic and affectively disordered patients appear to differ from normals. For example, psychiatric patients appear to have somewhat lower ratios of frontal to posterior cortical rates of glucose metabolism although the interpretation of these findings remain obscure. The ratios do not represent an absolute lowering of glucose metabolic rates in the frontal cortex of psychiatric patients. Schizophrenic patients who improve on neuroleptics show a further diminution in the normal anteroposterior gradient. Other preliminary findings suggest that schizophrenic patients have elevated glucose metabolism in both temporal lobes which appears to increase in the medicated state.

By electrophysiology, a diminution of the N120 component of the somatosensory evoked potential has been observed in normals in response to a series of similar somatosensory stimuli. This habituation which is most prominent in somatosensory area II does not appear to occur in schizophrenic patients, but appears normally in patients with affective disorders. This finding would appear related to our recent findings in the PET where the primary somatosensory area changes observed in normals appear to be diminished in schizophrenics.

OTHER PROFESSIONAL PERSONNEL

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 A. Catherine King, Psychology Technician, LPP, NIMH
 Susan Dowling-Zimmerman, Psychologist, LPP, NIMH
 Wayne Rasband, Computer Systems Analyst, RSB, NIMH

OBJECTIVE

The goals of this project are to develop and apply methods for imaging the brain based on its functional characteristics so as to further our understanding of normal and abnormal human behavior.

METHODS EMPLOYEDBehavioral Assessment

A detailed assessment and screening of all normal volunteers and patients participating in projects in the section occurs. This includes a structured interview, the Cannon-Spoor social adjustment scale, a detailed alcohol and drug history, family history and medical and psychiatric histories. Where appropriate, the Hamilton and Beck Depression scales, BPRS, the Strauss/Carpenter outcome scale, the global assessment scale, the AIMS (for motor movement ratings), and Krawiecka scale, to rate positive and negative symptom clusters in schizophrenia, are used. All subjects rate themselves using the Spielberger Anxiety Scale to report their experience during actual imaging procedures.

Biological Assessment

In conjunction with other laboratories subjects are often assessed for dexamethasone suppressibility, TRH responsiveness, serotonin platelet uptake, and drug treatment responsiveness. In addition, blood, urine and cerebrospinal fluid measurements reflecting neurochemical activity are in the future expected to be utilized in conjunction with electrophysiologic and PET data.

X-ray transmission tomography (CT Scan) is used for measurements of ventricular size, sulcal atrophy and hemispheric asymmetry. Positron Emission Tomogra-

phy (PET) is also used. PET, utilizing similar reconstruction mathematics as that of CT scanning, enables the user to obtain slice images of radioisotope cortical location. Using F^{18} -2-deoxyglucose (FDG) as the radioisotope tracer and the methods developed by Sokoloff and others, it is possible to obtain data on local glucose metabolism and consequently, probable local cortical functional activity.

Mapping of Electrical Activity

If a large number of electrodes are used, the recordings of EEG and evoked potential can also be used to develop images or maps of cortical activity, albeit of surface topology. Presently, 12 standard 10/20 system points on the left hemisphere and midline, and four additional points between existing posterior leads are used. This method offers the potential of tracking behavioral events in the millisecond range in comparison to the 30' of integrated cortical activity which the PET Scan displays.

MAJOR FINDINGS

Method Development

PET Scans result in a rate of glucose utilization. Methods for appropriate, accurate, and noninvestigator biased analysis of the enormous data accumulated by this method are required. Our analyses have progressed from using raw isotope density measures, to glucose metabolism rates using a 3 constant model, to what we hope is our final 4 constant model approach in which we adjust our calculated rates to take into account the rate of isotope dephosphorylation. That this appears to be a reasonable approach was born out by our statistical analyses. Using the 3 constant model a slice factor was observed in normals. When a 4 constant model is used, this disappears, presumably as a result of our accounting for the systematic bias introduced into the glucose metabolism rate measurement resulting from the consistent top down scanning procedure used.

As our chief interest in behavior-brain-relationships lie in gray-matter structures the initial attempts to develop analyses were with a peel technique described in more detail in prior annual reports, which looked at the outer gray matter mantle as determined on the basis of a specific distance from the skull. Currently, we are examining alternate methods of gray matter glucose rate determinations based on (1) looking for the maximum pixel value within a specified radial distance from the skull so as to try to compensate for partial voluming effects and (2) using region of interest approaches which either make use of thresholding algorithms or box templates which are designed in size and in application to minimize quantitative loss due to partial voluming and imprecise geometric fits.

One of the ideal methods to handle partial voluming effects is to have increased resolution. As many of the structures that one might be most interested in investigating in psychiatric patients involve the limbic system and since these structures are poorly resolved and even more poorly quantitated in our present ECAT scanner, an important part of what we can accomplish rests on our recent efforts to acquire a PET scanner with considerably higher resolution. Primarily through the efforts of Dr. Richard Coppola with the assistance of Dr. Larsen of the Nuclear Medicine Dept., we have critically analyzed the various possibilities available to us for the acquisition of a new high resolution, high

sensitivity scanner. As a result NIMH has ordered a scanner from Scanditronix that promises to deliver 5-6 mm resolution.

Furthermore, in anticipation of the rapid growth in our scans that would occur, were the acquisition and delivery of an NIH cyclotron to proceed on schedule for January, 1985, a major effort has been made to enhance the computerized support for PET and related projects; e.g., CT scan data, NMR, and EEG. To accomplish the goals of the section in assisting the entire NIMH research community with image analysis problems, a concerted effort is being made to establish a multi-user computer image analysis system that would host state-of-the-art color image/graphics processors. The available equipment on the market for this purpose has been carefully evaluated by John Cappelletti of our group with assistance from Wayne Rasband of the Research Services Branch, and contracts will be placed this year. Our EEG data acquisition capabilities are also being updated this year to allow us to make more meaningful comparisons of brain wave activity to glucose metabolism data.

Patient Studies with PET

Sixteen patients with schizophrenia, 11 patients with affective disorder and 19 normals have now had PET procedures during which they received somatosensory stimulation. All 3 groups demonstrated an anteroposterior gradient in glucose metabolism especially at superior slice levels. Both patient groups showed less of an anteroposterior gradient than the normals which represented primarily higher glucose metabolic rates in posterior regions, rather than lower levels in frontal regions. A 4-way ANOVA (groups by slice level by hemisphere by section) revealed no significant group or group interaction effects. Front-back ratios for right side were significant for groups only with 1-tailed t-tests. Normal subjects (1.08, S.D. = 0.11) vs. schizophrenics (1.02, S.D. = 0.08) was ($t = 1.78$, $p < 0.05$, 1 tailed) and for left side, the differences of 1.11 for normals vs. 1.06 for schizophrenics did not reach statistical significance. No significant differences were observed between affective disorder and schizophrenia groups.

Using an edge finding computerized program, schizophrenic patients were found to have elevated glucose use in both temporal lobes compared to controls; hallucinators were found to have greater ratios of left to right temporal lobe metabolism compared to non-hallucinators or controls. In addition to directing this latter effort, Dr. DeLisi has begun examining CAT scans to look for correlations with PET findings. So far, findings of hypofrontality in schizophrenia have not correlated with cerebral atrophy on CT Scans. Dr. DeLisi has also examined the effect of neuroleptic medication on these findings by comparing the PET scans of 9 schizophrenic patients medication free and during the medicated state. Preliminary findings suggest that the diminution of the anteroposterior gradient observed in nonmedicated schizophrenics is even greater in the medicated state despite the fact that most of the patients had at least partially recovered from their acute symptomatology. Similarly temporal lobe glucose metabolic rate increases were substantially enhanced over the pre-drug state as was the overall glucose metabolic rate. In general, we have been unable to establish any relationship between the degree of hypofrontality and symptomatology either in schizophrenia or in the affective disorders.

Although for the first 8 months of this year, we have been able to run less than 25 PET scans due to unprecedented difficulties in ^{18}F -deoxyglucose production, we have utilized these scans to introduce a cpt task. Our preliminary

analysis of this data suggests that this task may be particularly useful in differentiating affectively ill patients from normals.

One other effort appears worthy of mention. Because overall levels of glucose metabolism appear relatively unaffected in psychiatric illnesses and there is a relatively large variance in general metabolic rate, it appeared important to try to look for patterns of regional brain glucose metabolism; i.e., the pattern of correlations among brain regions. A substantial contribution to working out analyses along these directions have been made by Dr. Campbell Clark and Dr. Robert Kessler and now more recently by William Semple in our own group. To date there appears to be strong evidence that there is a normal pattern of relationships among different brain regions in normals undergoing somatosensory stimulation/electric shock that is reflected statistically by a very powerful factor in the Q profile analysis, which is a variant of factor analysis. This pattern should make it possible to investigate how different types of psychopathology as defined by DSM III or other diagnostic criteria affect this normal pattern. The approach also offers a promising tool for investigators to look for subclasses of patient groups within the present diagnostic nosology.

Human Studies with Electrical Mapping

Using a 16 lead evoked potential mapping system, Dr. Henry Holcomb has been directing the study of somatosensory responses to pain stimuli in normals, schizophrenic patients and affectively ill patients. This work has now progressed to include 23 normal volunteers, 18 patients with schizophrenia, and 12 patients with major depressive illness, all medication free. This work has been an important complement to the PET studies which make use of a similar paradigm and allow us to compare the two forms of brain imaging within the same subject. Each subject was given a somatosensory/pain discrimination task before and after a 32 minute period of noxious stimulation of the right forearm. During that epoch evoked cortical potentials were obtained from 16 scalp electrodes. Psycho-physical pain ratings administered prior to the shock period clearly distinguish normals from patients with schizophrenia, but do not distinguish normals from patients with affective illness.

Following mildly painful electrical stimuli of 16 mA for 1 msec duration, in normals there appears to be a gradual diminution in the N120 (the negative potential component of the somatosensory evoked potential occurring between 112-148 msec post-stimulation). There is a negative correlation ($r = -0.44$, $p < 0.05$) between this diminution and pain sensitivity as determined by a pain discrimination task. In comparison to the normals tested, unmedicated schizophrenic patients showed a constant value or sometimes a rise in this component. The level of habituation correlated significantly with BPRS ratings ($r = -0.57$). The differences in evoked potentials were most marked in what we believe to be somatosensory area II of the cortex. These differences among the groups appear also to be demonstrable in the PET, as normals and affectively ill patients, but not schizophrenic patients appear to show enhanced left compared to right somatosensory cortical glucose metabolism.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE

In the past, there have been considerable difficulties involved in trying to assess region specific functional activities as well as neurotransmitter functional activity in the brain. PET scanning and mapping based on electrical

activities provide two very exciting approaches for solving these methodologic problems. They promise to play particularly important roles in the study of psychiatric disorders where there is little evidence for structural changes in the brain. The findings so far have already begun to elucidate some aspects of the normal physiology of pain pathways and the possible psychiatrically related alterations in the same.

A concentrated effort has also been made to examine frontal cortex activation in normal and psychiatric patients as measured by the PET scan as the function of this anterior area of the cortex may relate more closely to observed alterations of behavior in psychiatric patients. In the past, the increased blood flow observed in this region upon task initiation has been hypothesized to result from the presumed responsibility of this region for the planning of goal-directed behavior in people in comparison to posterior cortical areas which may relate more directly to sensory processes. Based on this earlier work, however, we have tried to become somewhat more sophisticated and look at overall patterns rather than just the ratios of front to back. The capacity then to assess specific illness and task related deficits in activation provide us with an exciting and challenging tool for the elucidation of the mechanisms that might underlie abnormal behavior.

PROPOSED COURSE

We need to continue to improve our methods of statistical analysis. We appear to be headed in this direction with a variety of factor and discriminant analyses which should enhance our capacity to both discover group differences among patient populations as well as allow us to examine the homogeneity of the patient populations we do scan. We need to continue our efforts to improve the hardware and software available for data analysis of imageable data at NIMH as alluded to earlier. In this regard, we have hired a second person to do computer work and have on order a sophisticated hardware package. As the number of scans increase, the job of data management and analysis should become the rate-limiting step of the scientific process; but if we are not foresighted enough to prepare for this, it could become an insurmountable obstacle to progress in this field at NIMH. Our plans must include a multiuser system with capacities to utilize all the available imaging data on a patient; e.g., CT and NMR as well as PET, and software that would allow easy access and analysis of clinical investigator generated scientific questions.

There is the need to develop tests to assure that populations to be compared are actually being compared without artifact. For example, a continuous performance task in which you are more likely to be able to control the subject's cognition and have a means of assessing this control is likely to provide a good paradigm for intergroup comparisons and to establish adequate correlations between behavior and physiology. This has already begun this year and has provided preliminary data suggesting that the discovery of important PET findings may depend upon choosing the appropriate psychological task for the specific psychopathology to be examined.

We also need means to assess how dependent the glucography method is to the state of the patient at the time of scan. A protocol is currently under submission for examining state induced depression and euphoria in normals.

In evaluating the importance of PET data, most importantly, new tracers need to be developed to extend our functional mapping capacities. Just as the neurosciences have developed from anatomical concerns to neurotransmitter concerns, we

as the rest of the neurosciences must try to meld the two approaches of anatomy and biochemistry to give us neurotransmitter specific anatomical information on brain function, a task for which PET may be ideally suited.

In this regard, we are trying to add to our section Dr. Michael Chiueh, who has been developing in animals a new PET tracer fluorodopa which shows promise of being a significant dopamine specific radiotracer for use in man.

We need to continue to evaluate PET findings in relation to evoked potential data, biochemical measurements, and behavioral assessments including drug response. Insofar as only small differences have been observed between psychologically disturbed patients and normals, new strategies emphasizing selective neurotransmitter challenge approaches are a logical step in the search for neurotransmitter or regional functional differences between patients and normals.

In addition, the data on somatosensory habituation should be extended to study other patient groups; e.g., phobic patients and Alzheimer patients. The electrophysiological mapping procedures facilitate the study of the psychopharmacology of habituation, a very important fundamental process of the nervous system upon which most higher cortical functions may depend.

PUBLICATIONS

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Buchsbaum, M.S., DeLisi, L.E., Holcomb, H.H., et al.: Anteroposterior gradients in cerebral glucose use in schizophrenia and affective disorders. Arch. Gen. Psych. (in press).

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00508-02 LPP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neuropsychological Evaluation of Psychiatric and Neurological Patients

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Connie C. Duncan-Johnson, Ph.D. Chief, Unit on Psychophysiology LPP, NIMH

COOPERATING UNITS (if any)

Biological Psychiatry Branch, Clinical Neuroscience Branch, Laboratory of Clinical Science, Consultation Services, NIMH; and Epilepsy Branch, NINCDS; Albert Einstein College of Medicine; and Chestnut Lodge

LAB/BRANCH

Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

1.8

PROFESSIONAL:

0.2

OTHER:

1.6

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A comprehensive neuropsychological test battery has been devised to provide a complete assessment of various cognitive and sensory functions that can be related to damage or dysfunction in different regions of the brain. The battery comprises tests designed to tap the following aspects of behavior: executive functions, language, vigilance (attention), visual-spatial capacity, memory, and motor behavior. In addition, measures of psychometric intelligence, personality, visual acuity, color vision and hand and eye dominance are included. The battery provides an archival assessment of the neurobehavioral capacities of the various subgroups of patients who are studied by investigators within the LPP. The data thus provide a complete behavioral assessment against which to relate the neurophysiological, neuroradiological and biochemical information that is gathered concurrently on these patients. The data can also provide neurobehaviorally-defined subgroups that might reduce variability in psychiatric diagnosis, treatment, and outcome. The data are included in the permanent file of each patient and will provide eventual actuarial summarization of cognitive and perceptual functions for the different clinical populations that have been studied in the DIRP and facilitate research relating to behavioral factors in neuro- and psychopathology.

Other Professional Personnel

Allan F. Mirsky, Ph.D.	Chief	LPP, NIMH
Robert Post, M.D.	Chief	BPB, NIMH
David Pickar, M.D.	Chief	SCS, NSB, NIMH
David Jimerson, M.D.	Chief	SET, LCS, NIMH
David Rubinow, M.D.	Chief	BPB, NIMH
Roger Porter, M.D.	Chief	EB, NINCDS
Elkhonon Goldberg, Ph.D.	Associate Professor of Psychiatry	Albert Einstein College of Medicine
C. Wesley Dingman, M.D.	Assistant Clinical Director	Chestnut Lodge

Objectives

There are several goals of this project: (1) To provide a standard, comprehensive neurobehavioral assessment of all patients studied by the LPP for archival and actuarial purposes. As such, the data gathered form part of the permanent record for each patient and will facilitate current and future research relating to behavioral factors in neuro- and psychopathology. (2) To provide a complete behavioral description of various patients who are also being studied in conjunction with various research protocols but, more specifically, the protocols aimed at developing a taxonomy of attention disorders. The behavioral data can then be correlated with the neurophysiological, neuroradiological and biochemical data that are concurrently being gathered on these patients. (3) To provide neurobehaviorally-defined subgroups that might reduce variability in psychiatric diagnosis, treatment and outcome.

Methods Employed

The neuropsychological battery includes the tests listed below. Cognitive and sensory functions are presented in tabular form along with the test(s) used to assess them. Administration of the battery takes 8-12 hours.

FUNCTION MEASUREDTESTExecutive

Sequencing, Attention
Attention

Perception and Reasoning
Concept Formation and Abstraction

Trail Making Test
Stroop Color-Word Test
Cancellation Tests
Raven's Progressive Matrices (B-E)
Wisconsin Card Sorting Task
Halstead Categories Test

Language

Initiation
Lexical

Verbal Fluency Test
Boston Aphasia Test--Word
Discrimination and Visual
Confrontation Naming

Written	Boston Aphasia Test--Word Discrimination
Comprehension	Token Test Goldberg's Semantic Test
<u>Oral Apraxia</u>	Boston Aphasia Test--Oral Agility
<u>Vigilance</u>	Continuous Performance Test
<u>Visual-Spatial</u>	Raven's Progressive Matrices (A) Hooper Visual Organization Test Vitkin's Embedded Figures Test Butter's Embedded Figures Test
<u>Memory</u>	
Global	Weschler Memory Scale
Recent Verbal Memory	Buschke Selective Reminding Test Rey Auditory Verbal Learning Test Babcock Story Recall Test
Remote Verbal Memory	Boston Remote Memory Test
Recent Visual-Spatial Memory	Kimura's Recurrent Figures Test Rey-Osterreith Figure
<u>Motor Functions</u>	Purdue Pegboard Boston Apraxia Test
<u>General Intelligence</u>	Wechsler Adult Intelligence Scale- Revised
<u>Personality</u>	Minnesota Multiphasic Personality Inventory
<u>Sensory and Perceptual</u>	Visual Acuity Color Vision Hand Dominance Eye Dominance Kimura's Dichotic Medodies

Major Findings

The test battery has been administered to a total of 51 subjects, including 21 patients with eating disorders (7 hospitalized anorexics tested underweight and retested following weight recovery, 5 long-term weight-recovered anorexics and 8 bulimics); 7 normal subjects matched for sex, age, and education; 15 patients with complex partial seizures, and 3 patients with absence epilepsy.

Preliminary comparisons of 7 anorexic and 6 bulimic patients showed that on a test of sustained attention, the anorexic patients performed significantly ($p < .01$) poorer than the bulimics. This finding fails to support previous research showing that anorexics perform well on effortful tasks. On a test of general intelligence (WAIS-R), anorexics performed better on the Digit-Symbol

subtest ($p < .03$) and worse on the Comprehension subtest ($p < .005$) than the bulimic patients. The anorexics appear to have a deficit in visual-spatial ability relative to the bulimics ($p < .05$). The personality profile (as assessed by the Minnesota Multiphasic Personality Inventory) of both patient groups appears abnormal, as indicated by elevated scores on the majority of clinical scales. Anorexics showed more cognitive flexibility than bulimics ($p < .04$) on a test that assesses the ability to inhibit conflicting responses (Stroop Color-Word Test).

Two projects are underway on patients with affective illness. One project assesses neuropsychological performance as a function of mood state (depressed [or manic] vs. euthymic); the second assesses performance as a function of drug treatment (lithium vs. carbamazepine). Two subsets of tests from the battery that tap various aspects of attention, verbal and visual-spatial memory, problem solving ability, motor skills, personality, and the ability to estimate time (and are repeatable) were selected. The "mini-batteries" have been administered to nine patients and readministered to three patients. There are insufficient data to draw inferences at this time.

A short battery was devised to assess vigilance, attention, and memory in schizophrenic patients on a series of drug challenges. Data were collected on six patients before this collaboration was terminated. Because none of the patients completed testing, the data set is incomplete.

A 4-5 hour battery was developed to test a wide range of cognitive functions, including memory, concentration, and executive functions as well as mood, in patients with acquired immune deficiency syndrome (AIDS). Ten patients and three homosexual control subjects have been tested. Data analysis awaits testing of additional control subjects. These subjects will be retested on a subset of the battery six months following initial test administration and on the entire battery 12 months after initial testing.

Two tests, the Wisconsin Card Sorting Test and the Halstead Categories Test, which have long been considered to assess the same ability, namely, concept formation and abstraction, have been administered to 17 patients. In every group, regardless of diagnosis, the two tests appear to be tapping distinct cognitive abilities. The Categories Test appears to be related to verbal ability whereas the Wisconsin Card Sorting Test does not.

Significance to Biomedical Research and the Program of the Institute

The data base we are providing will constitute a permanent, archival behavioral assessment of the neuropsychological capacities of all patients seen by investigators in the LPP. It provides an invaluable resource for current as well as future studies in the LPP in which the goal is to relate behavior and physiology, whether pathological or normal. Of particular value and interest will be the correlations to be drawn in our taxonomy of attention research.

Proposed Course

We will continue to test all patients studied by investigators in the LPP to the extent that resources and time permit. As the sample size increases and data

accumulate, we will be able to construct neuropsychological test profiles for the different clinical populations under study and to begin to interrelate the behavioral, neurophysiological, biochemical, and neuroradiological domains of information. We plan to evaluate neuropsychological performance in schizophrenic patients when they are actively symptomatic and when they are in remission and to study the relatives of schizophrenic patients. If the success of the program warrants it, and if resources are available, the administration of the battery may be extended to include more DIRP patients.

Publications

None

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00509-02 LPP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Attention Disorders As Assessed by Event-Related Brain Potentials

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Connie C. Duncan-Johnson, Ph.D., Chief, Unit on Psychophysiology, LPP, NIMH

COOPERATING UNITS (if any)

Biological Psychiatry Branch, Laboratory of Clinical Science, Adult Psychiatry Branch, NIMH; Epilepsy Branch, NINCDS; and Chestnut Lodge

LAB/BRANCH

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SECTION

INSTITUTE AND LOCATION

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TOTAL MAN-YEARS:
2.2PROFESSIONAL:
0.9OTHER:
1.3

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The major purpose of this project is to investigate the roles of event-related brain potentials, attention and information processing and their interrelationships in the etiology, pathology and prognosis of psychiatric and neurologic disorders. Major emphasis is on the diagnostic specificity of disorders of attention and cognition and identification of the specific stage(s) of information processing underlying observed decrements in performance. Concurrently recorded event-related brain potentials and performance on cognitive tasks are used to define mechanisms of attention failure in subjects with diagnoses of schizophrenia, seizures, attention deficit disorder, learning disorders, eating disorders, dementing diseases, affective disorders, infantile autism and cerebral lesions. Biological processes influencing event-related brain potential activity are investigated by testing the effects of drugs and other treatments and by correlating these variables with biochemical measurements. Psychological correlates are investigated by relating the data to extensive neuropsychological, psychiatric and personality measures and performance on behavioral tasks.

Other Professional Personnel

Allan F. Mirsky; Ph.D.	Chief	LPP, NIMH
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Roger Porter, M.D.	Chief	EB, NINCDS
Elliot Gershon, M.D.	Chief	SP, BPB, NIMH
Judith Rapoport, M.D.	Chief	SCP, LCS, NIMH
C. Wesley Dingman, M.D.	Assistant Clinical Director	Chestnut Lodge

Project DescriptionA. Objectives

The major objective of this project is to yield data that will contribute to a taxonomy of attention disorders and to relate this to the clinical disorders of schizophrenia, epilepsy and other forms of brain pathology. Defining the specific ways in which information processing can fail may provide new diagnostic strategies for more effective evaluation and treatment of patients with attentional and cognitive impairments. A related objective of this project is to differentiate state vs. trait attributes of these disorders to increase understanding of their etiologies. Concurrently obtained event-related brain potentials and measures of performance during active cognitive processing are used to define the mechanisms of attention failure in these syndromes. Defining and understanding the different determinants and forms of attentional and cognitive failure is diagnostically important as well as useful in characterizing the nature of the psychology of attention disorders.

B. Methods Employed1. Electrophysiological Assessment

The general methods of these studies include recording the EEG, utilizing the International 10-20 system of electrode placement, while subjects perform a variety of tasks. Tasks include tests of auditory and visual attention and memory and use reaction time techniques and/or recall and recognition of stimulus material. The EEG is averaged to yield event-related brain potentials (ERPs). Since these electrical waves are associated in time with either an event in the environment, such as the presentation of stimulus, or with an internal cognitive event, they are called event-related potentials. ERPs provide information on the attentional and cognitive functioning of the subject. Using ERPs, it is possible to get an indication of the subject's processing of all environmental stimuli, both relevant and irrelevant, and thus to obtain, for example, the differential processing that is the hallmark of selective attention. A major focus of our investigations is the "P300" component of the ERP. This scalp-derived electrical potential appears approximately 300 milliseconds after an event that engages the interest or attention of a subject and is a positive voltage as recorded on the scalp, hence the name P300. The amplitude of the P300 component depends on the amount of information processing invoked by a stimulus and reflects stimulus evaluation and decision making activity. It is also a sensitive indicator of

orienting reactions to novel, surprising or incongruous stimuli and a predictor of the memorability of events. The latency of P300 indexes the duration of stimulus processing and decision making, independent of the time required for response processing. P300 latency also predicts the recognizability of events. This waveform is the focus of extensive investigative effort in our cognitive psychophysiology laboratory, as an index of brain activity that reflects attention and cognition. Stimulus presentation and data collection are controlled by a PDP-11/34 computer.

2. Neuropsychological Assessment

A detailed assessment is conducted on all patients and normal volunteers. This includes a standard structured interview that yields data on alcohol and drug history, family history and medical and psychiatric histories. Moreover, each subject is evaluated on an extensive neuropsychological battery of cognitive and sensory functioning. When appropriate, tests of formal thought disorder are administered.

3. Biological Assessment

In collaboration with other laboratories, subjects are assessed for drug treatment responsiveness. Blood, urine and cerebrospinal fluid measurements reflecting neurochemical activity will be correlated with electrophysiological and neuropsychological data.

We also plan to use X-ray transmission tomography (CT scan) to measure ventricular size and positron emission tomography (PET) to obtain data on local glucose metabolism. These data will be correlated with electrophysiological data to yield information on the relation between ERPs and the structure and functional activity of the cortex.

Major Findings

Considerable effort has been devoted to outfitting the ERP laboratory. This work has involved acquiring the requisite computer and related hardware, directing the development of the necessary system and paradigm software, training technicians and creating both between- and within-institutional liaisons needed to acquire patient and control subjects. The laboratory is fully operational and pilot work (on 16 normal control subjects) is complete. A number of clinical studies are under way, including psychophysiological investigations of schizophrenia, absence epilepsy and eating disorders (anorexia and bulimia).

A battery of attentional paradigms, which tap visual and auditory information processing systems, has been developed. Processing dysfunctions are considered from the paradigmatic perspective of an experimental psychology of how the subjects process information in a variety of tasks. The battery provides a differential assessment of specific types of attention, including the ability to initiate, select, inhibit, shift and sustain attention. The protocol also includes evaluation of automatic and controlled cognitive processes. The rationale for the approach of using a battery of tasks that tap specific cognitive processes is to allow inferences about which processes are uniquely

impaired in one group in comparison to other groups. To determine whether ERPs can serve as sensitive yet specific markers of disorder, patients with diverse symptomatology and diagnoses are compared. The ERP measures are correlated with concurrently recorded behavioral responses, including reaction time, as well as performance on the LPP Neuropsychological Battery and tests of formal thought disorder.

Six unmedicated schizophrenic patients have been tested in 2-3 experimental sessions each. Three patients with absence epilepsy have completed five experimental testing sessions. Five underweight anorexics, one weight-recovered anorexic and two bulimic patients have been studied to date. Moreover, five normal controls have been tested. Preliminary analyses indicate that patients with schizophrenia show that the P300 elicited by auditory stimuli is reduced in amplitude in schizophrenic patients relative to normal subjects. Because of the small number of subjects tested, the consistency of this result is uncertain at present. Moreover, additional normal control subjects are needed. The data do, however, suggest altered cerebral mechanisms underlying attentive/cognitive behavior in schizophrenia, absence epilepsy and anorexia. More specifically, there is evidence that the underlying pathology may involve auditory information processing preferentially in schizophrenia and anorexia and visual information processing in absence epilepsy. There are insufficient data on the effects of weight recovery on the cognitive processing of anorexics to draw any inferences at this time.

Significance to Biomedical Research and the Program of the Institute

Since attention and cognitive deficit are characteristic of many prominent psychopathological and neuropathological disorders, it is important to develop a precise empirical and theoretical account of these symptoms. The scalp-recorded ERP is the only noninvasive approach available to study rapidly changing neural activity associated with cognitive processing in human subjects. The ERP provides information on mental events involved in selective attention, stimulus evaluation and decision making, memory, learning and response preparation. For example, the latency of one ERP component, the "P300," has been shown to index the duration of cognitive processing and decision making, independent of the time required to make a response. The temporal resolution of ERPs can support inferences about brain activity on time scales not possible in studies using tissue assays or radioactivity. Because of the noninvasive character of P300, patient state can be monitored often enough to assess the effects of specific clinical or experimental variables. The appropriateness of evaluating ERPs in studies of attention is apparent, as they may provide a dissection of the various components involved and thereby permit more precise identification of the type of information processing deficit responsible for poor performance on attention tasks in a variety of patient groups. Moreover, because ERPs can provide information independently of overt responses, they are especially useful for studying patients in whom overt behavior may be altered or impaired.

Proposed Course

Collection of data will continue for projects on schizophrenia, epilepsy and eating disorders. Planned investigations will address disorders of attention and

memory in adults and children with attention deficit and learning disorders and patients with progressive idiopathic dementia. This work is aimed at illuminating the neuropsychological bases of the cognitive-attention deficits in these patient groups. The goal is to determine the relation of ERP variables to diagnosis, diagnostic symptomatology, severity of psychosis, degree of formal thought disorder, performance on tests of attention and memory and intellectual functioning, degree of improvement during treatment and improvement on specific treatments. To increase our understanding of the etiology of schizophrenia, we are planning studies to differentiate state vs. trait attributes of the disorder. One strategy we intend to use is to compare normal controls with schizophrenic patients when they are actively symptomatic and when they are in remission. A second strategy, aimed at yielding information on the hereditary nature of the disorder, is to study the relatives of patients. The latter investigation is planned in collaboration with the Section on Psychogenetics. Selected studies with brain-injured cases are planned to test hypotheses derived from various clinical groups concerning the involvement of brain structures in the pathophysiology of psychiatric disorders. ERP measures in patient groups will be studied in relation to data obtained from CT and PET scans as well as biochemical assays of body fluids such as monoamines and their metabolites in cerebrospinal fluid. Electrophysiological predictors of clinical response to neuroleptic medications will be sought. The ERP data-collection facilities are currently being expanded to accommodate the proposed collaborative investigations.

Publications

Duncan-Johnson, C.C., Roth, W.T., and Kopell, B.S.: Effects of stimulus sequence on P300 and reaction time in schizophrenics: A preliminary report. In Karrer, R., Cohen, J., and Tueting, P. (Eds.): Cognitive Processing and Brain Activity. New York, The New York Academy of Sciences, in press.

Duncan-Johnson, C.C.: P300 applications to research on schizophrenia. Electroencephalography and Clinical Neurophysiology, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00672-19 LSES

PERIOD COVERED

October 1, 1983, to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Social Psychological Correlates of Occupational Position

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: M. L. Kohn, Chief, Laboratory of Socio-environmental Studies, NIMH

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COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Socio-environmental Studies

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

11.00

PROFESSIONAL:

5.00

OTHER:

6.00

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The object of this study is to assess the reciprocal effects of occupational conditions and psychological functioning (in particular, values, self-conceptions, social orientation, and intellectual flexibility). Structured interviews were conducted in 1964 with a sample of 3101 men, representative of all men employed in civilian occupations throughout the United States. The study was extended into a longitudinal study in 1974, with the reinterviewing of a randomly-selected one-fourth of the original sample, together with their wives and, where appropriate, one of their children. Replications of this research have been carried out in Poland and Japan.

Project Description:

The principal goal of this research is to assess the relationships between people's job conditions and their psychological functioning. The evidence thus far provided by this research demonstrates that job conditions have a marked impact on cognitive functioning, on values, and on conceptions of self and orientations to society. Psychological functioning, in turn, has a rather more gradual but substantial impact on job conditions.

The research began in 1964 with structured interviews with a sample of 3101 men, representative of all men employed in civilian occupations throughout the United States. These interviews were conducted to Melvin Kohn and Carmi Schooler's specifications by the National Opinion Research Center (NORC) of the University of Chicago. In 1974, NORC conducted follow-up interviews, again to Kohn and Schooler's specifications, with a randomly selected one-fourth of the men who had participated in the original survey. Wherever a man was found to be presently married, a nearly identical interview was separately conducted with his wife. And wherever a man had one or more children in the age-range 13 through 25, a similar interview was conducted with a previously selected child.

One major purpose of the follow-up study has been to provide more definitive data about causal processes than could be provided by a single cross-sectional survey. With these data, the investigators have assessed the magnitudes of the reciprocal effects of job conditions and several important facets of psychological functioning. The study of wives was designed to ascertain whether job conditions affect men and women similarly. The research has shown that they do. The study of the children was designed for exploratory analyses of the effects of parental experiences, values, and practices on their children's psychological development, as well as of children's educational and occupational experiences on their own psychological development.

During the current year, the major research efforts have been addressed to the following activities: an analysis of the processes by which values are transmitted from one generation to the next; further analysis of the relationship between educational experience and children's personality development; the beginning of an analysis of intra-family dynamics and children's personality development; an assessment of the impact of husbands' and wives' social statuses on wives' psychological functioning; and continued cross-national comparative analyses, for Poland and Japan, of the relationships between job conditions and adult

psychological functioning. This Annual Report will summarize all of these activities.

THE INTERGENERATIONAL TRANSMISSION OF VALUES

The goal of Melvin Kohn, Kazimierz Slomczynski, and Carrie Schoenbach's analysis is to redefine a classic sociological problem -- the transmission of values in the family. They see the transmission of values in the family as part of a much more general process by which social structure -- in particular, social stratification -- affects children's values and orientations, with the family as only one -- albeit a crucial -- institutional mechanism in this process.

By values they mean conceptions of the desirable -- criteria of preference. By parental values, they mean the values that parents would most like to see embodied in their children's behavior -- the characteristics they consider most desirable to inculcate in their children. Parent-to-child value transmission implies, at minimum, that children come to hold the same fundamental values for themselves that their parents think desirable for them. As described in last year's Annual Report, Kohn, Slomczynski, and Schoenbach began their analysis of the process of value transmission by developing confirmatory factor-analytic measurement models of parents' and children's values, using data from representative samples of parents and children in the United States and Poland. In both countries, the focus is on valuation of self-direction versus conformity to external authority. From these analyses, it appears that the correlations between parents' and children's valuation of self-direction/conformity are considerably stronger than past studies would have led one to expect. For the United States, using longitudinal data for fathers and considering values for children across the entire age-range of 13 to 25 years of age, they found the correlation between fathers' and children's values to be .58 and that between mothers and children to be .52. The analysis for Poland uses cross-sectional data and deals with children in the younger half of that age-range. The correlation between mothers' and children's valuation of self-direction is nearly identical in Poland ($r=.55$) to what it is in the United States. The correlation between fathers' and children's values, however, is lower for Poland ($r=.37$) than for the United States. It is not yet known whether this is a true cross-national difference or a methodological artifact -- since the U.S. analyses not only deal with a much broader age-range of children, but also are based on longitudinal data for fathers, which may strengthen the measurement properties of the index of paternal values. This is an issue that will be explored further.

Even correlations of .58 and .52 leave much to be explained, with fathers' and mothers' values together "accounting for" less than half of the variance in children's values. Moreover, calculating the correlations between parents' and children's values is only the first step in the

analysis of the intergenerational transmission of values. Even a very high correlation between parents' and children's values does not necessarily mean that parents' values have been transmitted to their children. A full analysis requires the development of causal models that assess the degree to which social stratification affects children's values through its effects on parents' values (then transmitted from parents to children) and the degree to which social stratification affects children's values (if it does) through other processes. Answering this very general question requires answering the following constituent questions:

1. What are the contributions of husband's and wife's educational attainments, occupational statuses, and incomes to the social stratification position of the family? It has become clear that simply using the status of the husband as a measure of family stratification position is insufficient, but there is no agreed-upon method for assessing the contributions of husband's and wife's statuses to the stratification position of the family.

2. Does higher family stratification position result in children's valuing self-direction more highly? If so, what is the magnitude of the relationship between family stratification position and children's values? -- Is it trivial, or does social stratification have a substantial relationship to children's values?

3. To what extent does the relationship between family stratification position and children's values result from stratification affecting parents' values, which in turn affect children's values? (It could be that social stratification affects children's values primarily through the influences of neighborhood and school; parents' values are not necessarily critical to this process.) If the process does involve parents' values, is it mothers' values, fathers' values, or both parents' values that is involved?

4. Assuming that social stratification does affect parents' values, what is the process? (Here the investigators lean on their earlier studies of U.S. and Polish men, which showed that occupational self-direction is of pivotal importance for explaining the impact of social stratification on values.)

5. What is the relationship between husbands' and wives' values? Do husbands' values affect their wives' values, do wives' values affect their husbands' values, neither, or both?

6. Is the relationship between parents' values and children's values unidirectional, from parents' values to children's values, or are the effects reciprocal, with parents' values both affecting and being affected by their children's values?

7. Insofar as parents' values do affect children's values, is it necessary for children's perceptions of their parents' values to be accurate?

8. Finally, are all the foregoing processes essentially similar for male and female children?

In further work this year, the investigators have made significant progress in answering the first five of these questions. In dealing with the first question -- the measurement of a family's social stratification position -- they explored several alternative methods of measurement, based on different conceptions of how husbands' and wives' statuses might contribute to the overall social position of the family. The most satisfactory model was produced by the method that made the simplest assumption: namely, that the position of the family in the social stratification system is best inferred from the covariation of husbands' and wives' educational levels, occupational statuses, and incomes. Technically, the model of family social stratification position developed for the U.S. is a third-order confirmatory factor-analytic model. The first-order components are multiple-indicator models of each partner's occupational status (based on several alternative indices of occupational status), together with less complex indices of educational attainment and job income. The second-order components combine husband's occupational status and income into a single concept, his occupational position; similarly for wives. Finally, the third-order component posits an underlying concept, the social stratification position of the family, inferred from the covariation of husband's educational level, husband's occupational position, wife's educational level, and wife's occupational position.

In the model for the U.S., the investigators find that husband's educational level and occupational position contribute somewhat more to the family's overall social stratification position than do wife's educational level and occupational status, but that the wife's statuses do make a substantial contribution to the family's overall position. [The standardized paths from family social stratification position to the four statuses -- which are analogous to "loadings" in an ordinary factor analysis -- are .84 for husband's education, .83 for husband's occupational position, .68 for wife's education, and .63 for wife's occupational position (limited to employed wives).] The model for Poland is constructed somewhat differently, mainly because data on income are available only in the form of combined family income -- but the results are similar. The only notable difference is that, for Poland, wife's education makes as much of a contribution to family social-stratification position as does husband's -- the standardized paths being .80 and .81, respectively. [The other components of the model are husband's occupational status, for which the standardized path is .73, wife's occupational status, .68, and family

income, .52.] In both countries, then, there is very substantial covariation of husbands' and wives' statuses. It is meaningful to think in terms of the family's position in the social stratification system. It is possible to index that position rigorously.

In further analysis (Question #2), the investigators found that family social stratification position, so indexed, is substantially correlated with children's valuation of self-direction versus conformity to external authority. For the United States, this correlation is .54; for Poland it is .31. (Here again it is not yet certain whether an apparent cross-national difference is real or is a statistical artifact. One can be certain, though, that in both countries the correlation is substantial.)

The real question, still, is not the magnitude of the correlation between family social stratification position and children's values, but the explanation for this correlation (Question #3 above). In terms of causal modelling, this question becomes: To what degree does family social stratification position affect children's values through social stratification affecting parents' values and parents' values, in turn, affecting children's values; and to what extent does social stratification affect children's values through processes other than parents' values, e.g., through the influences of school and neighborhood? For the U.S., about two-thirds of the total effect of social stratification on children's values is indirect, through parents' values. Fathers play a somewhat larger role in this process than do mothers, both because fathers' values are somewhat more affected by social stratification than are mothers' values, and because fathers' values have a somewhat greater effect on children's values than do mothers' values. The key finding, though, is not the small difference in the importance of mothers' and fathers' roles, but that the effects of social stratification on children's values is mainly through parents' values, with fathers playing at least as large a part in this process as do mothers. The Polish model gives results that are similar in showing the effects of social stratification on children's values to be mainly -- in fact, entirely -- through parents' values. The Polish model differs from the U.S. model, though, in showing this process to occur more through mothers' than through fathers' values. (For the reasons stated above, it is not yet certain whether this is a true cross-national difference or a methodological artifact.) In any case, the crucial finding for Poland as for the U.S. is that parents' values play a key role in the effects of social stratification on children's values.

The next issue (Question #4 above) is to account for the effects of social stratification on parents' values. Here the investigators built on their prior analyses of U.S. and Polish men, testing anew the hypothesis that, to a very substantial effect, stratification affects parental values because parents' social stratification position affects their occupational self-direction and occupational self-direction in turn affects their values for children. As in past analyses, there are three stages to testing this

hypothesis: First, the investigators developed models that establish the close relationship between social stratification and occupational self-direction. (These models show a strong unidirectional effect of education on occupational self-direction; they also show strong reciprocal effects between occupational position and occupational self-direction.) Second, the investigators found that the (unidirectional) effects of education are to some substantial degree, and of occupational position are almost entirely, indirect, through their effects on occupational self-direction, which in turn affects parents' values. Third, and crucially, they demonstrated in reciprocal-effects models that occupational self-direction does have an actual causal effect on parents' values. These analyses hardly come as a surprise, for all three models had been developed and tested in the investigators' prior analyses of these same data. These models do advance our knowledge, however, in two respects: The analyses are somewhat more precise than earlier analyses (being based on refined indices and full-information modelling); and the analyses have now been applied to working mothers in the U.S. and Poland, with results quite similar to those earlier found (and now reconfirmed) for U.S. and Polish fathers. The analyses, demonstrate, then, that for both fathers and mothers, in both the United States and Poland, social stratification affects parental values primarily through its effect on occupational self-direction, which in turn affects parents' values.

The investigators are currently working on the fifth question: the relationship between fathers' and mothers' values. The model is exceedingly complex, for it simultaneously includes the reciprocal effects of fathers' occupational self-direction and their own values, mothers' occupational self-direction and their own values, and fathers' values with mothers' values. Testing three pairs of reciprocal effects in one causal model is a difficult enterprise, one that, so far as we know, no one had previously succeeded in accomplishing. The investigators have solved such a model for the U.S., with these results: Mothers' values have a modest, albeit statistically significant, effect on fathers' values (the standardized path = .08); fathers' values have an even more modest, statistically nonsignificant, effect on mothers' values (path = .05). Essentially, each partner's values are derived from his or her own educational and occupational experiences, with little or no influence of either spouse's values on the other's. The investigators are now in process of developing a similar model for Poland. Analyses designed to answer Questions #6-8 are not yet underway. These constitute the agenda for the coming fiscal year.

EDUCATIONAL EXPERIENCE AND CHILDREN'S PSYCHOLOGICAL DEVELOPMENT

The purpose of Karen Miller, Melvin Kohn, and Carmi Schooler's analysis, now nearing completion, is to examine the processes by which students' educational experiences, particularly the degree of

self-direction they exercise in their educational endeavors, affect their psychological functioning. Data for this analysis were collected in the 1974 follow-up survey, when one pre-selected child of each father in the sample was interviewed. The interview schedule for these "children" -- by then aged 13 to 25 -- contains an intensive battery of questions about the current educational experiences of all those respondents still in school. These questions, designed to parallel those found to be useful for analyzing adults' occupational experience, focus on such dimensions of the educational experience as its substantive complexity and how closely it is supervised. The underlying hypothesis is that self-direction is important in young people's school experiences, just as in older people's job experiences. People who use initiative, thought, and independent judgment in their daily work, whether in school or paid employment, come to be more effective in their intellectual functioning, to have more self-directed orientations and values, and to be less distressed.

Empirical assessment of this hypothesis is hampered by the lack of longitudinal data. It is therefore not possible to statistically control earlier levels of psychological functioning in assessing the impact of educational self-direction on current psychological functioning, nor to statistically control earlier levels of educational self-direction in assessing the impact of psychological functioning on current educational self-direction. The investigators do, however, have one great advantage in this analysis: They have information about the psychological functioning of all of the students' fathers and most of their mothers. This means that, in assessing the impact of educational self-direction on the psychological functioning of students, they can statistically control parental psychological functioning. This makes it possible to take into account, to some substantial degree, family-experiential and genetic determinants of psychological functioning. A model of the reciprocal effects of educational self-direction and any facet of psychological functioning can thus include the corresponding psychological characteristic of each of the parents, as well as students' ages and grade levels, the extent to which school courses are compulsory or elective, and pertinent social characteristics of the students and their families.

Last year's report described a model for one important facet of psychological functioning -- ideational flexibility, the major aspect of cognitive functioning measured in this research. The main conclusion was that educational self-direction and ideational flexibility have substantial, approximately equal, reciprocal effects. The impact of educational self-direction on ideational flexibility results mainly from the substantive complexity of students' schoolwork -- its scope, difficulty, and challenge. During the current year, the investigators re-evaluated this model in light of an important question: the extent to which the findings are dependent on the assumptions made in estimating the model. Any reciprocal-effects model requires that certain assumptions be made -- usually, that some effects can only be indirect -- because

otherwise there is insufficient information to disaggregate a single correlation into two reciprocal paths. This problem, known as the "identification problem", is even more thorny in cross-sectional analyses than in analyses using longitudinal data; in the absence of longitudinal information, there are less certain grounds for assuming that some effects can only be indirect. To determine the robustness of the results when these assumptions are relaxed, the investigators performed a series of tests, relaxing each of the assumptions in turn. The model proved to be quite robust, showing that, whatever assumptions were employed, educational self-direction always has a positive, statistically significant effect on ideational flexibility.

Last year's report also described preliminary versions of causal models of the relationship of educational self-direction with self-directedness of orientation and with distress. This year, these models were extensively refined and their robustness confirmed. Educational self-direction leads to a more self-directed orientation; this results almost entirely from the substantive complexity of schoolwork affecting self-directedness of orientation. The reciprocal effect, from self-directedness of orientation to educational self-direction, is positive but not statistically significant. The impact of educational self-direction on distress is, as expected, negative and substantial. Close supervision strongly increases distress, while substantively complex schoolwork decreases it. In the reciprocal causal direction, more distressed students are significantly less likely to exercise self-direction in their schoolwork. Thus, while educational self-direction affects both self-directedness of orientation and distress, the causal mechanisms are quite different for these two aspects of psychological functioning.

The investigators examined two other variables that they thought might be related to educational self-direction. First, they tried to develop a measurement model of leisure-time intellectuality, analogous to one previously developed for adults. The effort failed, because the intercorrelations among leisure-time activities are much weaker for students than for adults. This may be because much of students' leisure time is spent in school-related activities. In any case, the question of the impact of educational self-direction on leisure-time intellectuality cannot be resolved with these data. The second new effort, which proved successful, was to analyze the relationship between educational self-direction and valuation of self-direction versus conformity to external authority. The impact of educational self-direction on valuing self-direction is, as expected, substantial and positive. This results mainly from the positive effect of the substantive complexity of the schoolwork, but closeness of supervision by teachers also has a modest, statistically significant, negative effect on valuation of self-direction. In the reciprocal causal direction, self-directed values have a positive but statistically nonsignificant effect on the exercise of educational

self-direction. The causal model for values, like those for other aspects of psychological functioning, was tested for robustness; the results proved to be highly robust, consistently showing a positive, statistically significant impact of educational self-direction on self-directed values.

The investigators are now attempting to build a larger causal model encompassing the reciprocal interrelationships of educational self-direction, intellectual functioning, self-directedness of orientation, and distress. Such a model, if successful, will show not only the independent effects of each variable (with all the others statistically controlled), but also the processes by which these effects occur. In particular, the model is designed to show whether educational self-direction directly affects all four aspects of psychological functioning, or whether, for example, educational self-direction affects ideational flexibility and distress partly through its effect on self-directedness of orientation, as is the case for adults' occupational self-direction. Successfully developing the expanded model, though a complex task, should greatly expand our knowledge of the relationships between educational experience and psychological functioning.

Whatever the larger model shows, the analyses already completed have demonstrated that the "quality" of the educational experience does make a difference for students' psychological development. The substantive complexity of schoolwork is primary in importance; more substantively complex schoolwork leads to more effective intellectual functioning, to a more self-directed orientation, and to greater valuation of self-direction. Closeness of supervision by teachers affects neither intellectual functioning nor self-directedness of orientation, but does have a modest, statistically significant effect on values. Moreover, closeness of supervision, rather than the substantive complexity of schoolwork, is what affects distress: the more closely supervised, the more distressed is the student. These findings parallel what has previously been found for adults in the workplace and thus extend our knowledge of how work and personality affect one another in different institutional settings and at different stages of the life-course. In particular, they show that not only for adults, whose work is paid employment, but also for children and young adults, whose work is schooling, the experience of self-direction in work is important for psychological functioning.

INTRA-FAMILY DYNAMICS AND CHILDREN'S PERSONALITY DEVELOPMENT

This year Carmi Schooler began working on a complementary approach to the study of intergenerational effects within families -- an investigation of the ways that reported child-rearing practices and family relationships affect children's psychological functioning. In its initial stages, this analysis involved substantial recoding of interview responses. A major reorganization of the data files was also required, so that the data from

fathers', mothers', and childrens' interviews could be analyzed together. Once these tasks were accomplished, confirmatory factor analysis was used to develop measures of the many aspects of parent-child relationships covered by the interviews. Indices were developed of such aspects of parents' relationships with their children as strictness, warmth, and degree of dominance over the child, as well as such measures of the children's responses to their parents as the degree they feel free to talk things over with each parent and the likelihood they will turn to each parent when troubled. The data have now been arranged in the form of a correlation matrix, necessary for the next step in the investigation -- the use of linear structural-equation analysis to explore the ways that parental practices and parent-child relationships affect the psychological functioning and values of children.

EFFECTS OF HUSBAND'S AND WIFE'S SOCIAL STATUS ON PSYCHOLOGICAL FUNCTIONING

Carrie Schoenbach has used the followup data of the U.S occupations study to test the validity of a commonly-held assumption in social-psychological research -- that the social stratification position of a family is defined by the status of the husband (using such measures as his education, income, and occupational status). It becomes critical to examine this assumption when we ask about husband's and wife's psychological functioning in families where both spouses are employed because, as research in this Laboratory has established, social stratification position matters for psychological functioning primarily because of the impact of occupational conditions, which are systematically associated with social stratification position.

Schoenbach's analysis asks whether the personality characteristics of employed wives are, in fact, related more to their husbands' social stratification positions or to the women's own positions. The characteristics examined are self-directedness of orientation, distress, and intellectual flexibility. The model uses linear structural-equations analysis to estimate the direct effects of each partner's education, occupational status, and income on a particular aspect of his or her spouse's personality, while also measuring the indirect effects through one's own status affecting one's own personality, which in turn affects the spouse's personality. The effects of status on personality are examined separately for each aspect of personality. The consistent finding is that, for wives as well as for husbands, it is one's own social stratification position that is of predominant importance for personality.

THE POLISH REPLICATION

The main purpose of the Polish replication has been to see whether the interrelationship of social stratification, job conditions, and psychological functioning are similar in socialist and capitalist

societies. Three principal co-investigators, Kazimierz Slomczynski, Krystyna Janicka, and Jadwiga Koralewicz-Zebik, carried out in 1978 in Poland a precise replication of the survey originally conducted by Kohn and Schooler in 1964 in the United States. After the data had been collected, coded, and edited in Poland, Slomczynski brought them to NIH, where he, Joanne Miller, and Melvin Kohn have been analyzing them. Previous Annual Reports reviewed the development of methods designed to assure cross-national comparability of indices and the analysis of two of the central questions of the Polish replication: Do people's positions in the system of social stratification bear the same relationships to their values and orientations in socialist Poland as in the capitalist U.S.? If so, do these relationships result from the greater opportunities for occupational self-direction enjoyed by men of higher social-stratification position? As reviewed in detail in earlier Annual Reports, the answers to both questions are positive with respect to values and social orientations, but not with respect to self-conception.

Further comparative analysis of the Polish and U.S. data has focused on social stratification and the intergenerational transmission of values (discussed above) and three further questions.

(1) The first question is whether the relationships between occupational self-direction and intellectual process are similar for younger, middle-aged, and older workers. Recent work in the developmental and social psychologies of aging suggests that learning, particularly as represented in "crystallized intelligence," continues throughout the life-span. In principle, since "transfer of learning" is an essential characteristic of the learning process, not only initial learning but also the generalization of what has been learned should continue as workers grow older. It is nevertheless possible that learning-generalization does not occur at the same rate or to the same extent at all ages and all stages of career and life-course. The process may be especially pronounced in younger workers, at early stages of their occupational careers, and before they are preoccupied with family responsibilities, but may diminish as workers grow older, advance in their careers, and have changing family responsibilities. It is also possible that either learning or generalization diminishes as workers grow older, simply because of biological decrements. To see whether learning and the generalization of learning continue unabated throughout adult life requires an analysis of how job conditions affect the psychological functioning of workers at different ages, or stages of career, or stages of life-course. Joanne Miller, Kazimierz M. Slomczynski, and Melvin Kohn have attempted such an analysis. They believe that this analysis is the first systematic empirical effort to see whether learning-generalization continues to be responsive to the social-environmental conditions of natural settings as workers grow older.

The analysis is cross-national: It examines the continuity of learning-generalization in two countries, Poland and the United States. As in any analysis of the relationship between social structure and personality, cross-national comparative analysis has the utility of ascertaining whether the findings in any one country are specific to the culture and to the economic and political system of that country. An analysis of people of differing ages offers the further advantage that even the "same" cohorts have had different experiences in different countries. When the two countries are Poland and the United States, we also have the opportunity of seeing whether the findings are consistent for a socialist and a capitalist society.

For both Poland and the United States, the investigators did separate analyses of job conditions and intellectual process for younger, middle-aged, and older men. For each country, their analysis asked: Is the relationship of job to intellectual process consistent for all three age-groups? Since a rigorous test of between-group similarities should allow the possibility that men of differing age-groups vary in how their answers to the interview questions relate to the underlying concepts one wishes to measure, the analysis began with the construction of separate measurement models of all major concepts for the separate age-groups. Fortunately for ease of analysis, the measurement models proved to be very similar for all three age-groups in both countries.

The main conclusion of these analyses is that job conditions affect intellectual process in older men just as much as in younger men. In particular, job conditions facilitative of the exercise of self-direction in work continue to enhance ideational flexibility and an open-minded, non-authoritarian orientation, even in the "oldest" segment of the workforce, that is, among men aged 46 to 65 years. These findings emerge both in cross-sectional and in longitudinal analyses for the United States. For Poland, only cross-sectional analyses are possible; these analyses do indicate that the findings apply as well to that country. What makes the investigators confident, albeit not certain, that their conclusion applies as well to Poland is the consistency of the U.S. longitudinal and cross-sectional findings. Learning and generalization from the experiences of work occur regardless of age, and -- if one may extrapolate -- regardless of stages of career and life course.

Moreover, the investigators have discovered notable continuity not only of the effects of job conditions on intellectual process, but also of the effects of intellectual process on job conditions -- a continuity of reciprocal process into later stages of career and later stages of life course. Men continue to learn from their jobs and to generalize those lessons to outside-of-job reality, and men continue well into their careers to select and to mold their jobs to fit their intellectual proclivities.

Finally, one can consider the findings of this analysis from the perspective of what they tell us about the generality of the effects of occupational self-direction on psychological functioning. These findings add substantially to the body of evidence that supports the generality of those effects. The present analysis shows that occupational self-direction, particularly the substantive complexity of work, has a decided effect on ideational flexibility in Poland -- a new and important piece of evidence. More generally, the findings tell us that the relationship between occupational self-direction and intellectual process holds for six groups of men who have had decidedly different generational and historical experiences. The generality of the relationship is thus extended not only with respect to age, but also with respect to diversity of experience.

(2) Krystyna Janicka (Polish Academy of Sciences), Grazyna Kacprowicz (University of Warsaw), and Kazimierz Slomczynski continued their analysis of the Polish data, to evaluate the reliability of the measurement model of occupational self-direction. They found the multiple correlation between the overall index of the substantive complexity of work with the independently coded ratings of the complexity of work with things, with data, and with people given in the Polish version of the Dictionary of Occupational Titles to be 0.81. They then went on to assess the validity of the index by comparing it to evaluations of job complexity based on much more detailed descriptive information about job content. To do this, they re-interviewed subsamples of their national sample in two industrial cities, Lodz and Wroclaw. These interviews provided more detailed information about job content than it would be possible to obtain in any broader survey. Using a number of alternative coding schemes, Janicka, Kacprowicz and Slomczynski found high correlations (in the .90's) between the index of substantive complexity of work based on brief and on detailed descriptions of the job. These investigators found also that direct (subjective) questions about the complexity of work provide only biased appraisals of the substantive complexity of the work and cannot substitute for descriptive questions about the actual content of the job.

The index of complexity of work has been compared to two indices of occupational position: an index of educational requirements for the job and an index of occupational prestige. Slomczynski found that the index of complexity of work is more powerful than the other indices in terms of explaining the status-attainment process. In particular, if all three indices are included in a multiple-indicator structural equation model of the status-attainment process, the index of complexity of work has the greatest effects. Over the course of the occupational career, the complexity of work becomes increasingly important, while the effects of educational requirements and occupational prestige diminish.

(3) Slomczynski carried out an analysis of the effects of status inconsistency on psychological functioning, using the Polish data. He used

formal education, occupational prestige, and job income as indices of social stratification. His major assumption is that status inconsistency should be separated from status per se, by methods of measurement that assure the orthogonality of the constructs. Accordingly, status is conceptualized as the weighted sum of education, occupational prestige, and income, while status inconsistency is conceptualized as the weighted difference between pairs of the same indices of stratification. Slomczynski adapted principal component analysis to obtain an orthogonal solution for both constructs. Since status is, by definition, the first component, its effects on psychological functioning must be greater than those of status inconsistency. Still, the question remains whether the psychological effects of status inconsistency are statistically significant and non-trivial in magnitude.

Significant effects of status inconsistency were found in the domain of intellectual process, measured by ideational flexibility and authoritarian conservatism, as well as in the domain of social orientation, in particular, trustfulness and personally responsible standards of morality. Ideational flexibility linearly decreases while authoritarian conservatism increases with the extent to which education and prestige are disproportionately high relative to income. Trustfulness and personal standards of morality decrease with status inconsistency, especially if income is disproportionately low. Status inconsistency adds substantially to the overall explanation of the variability of psychological constructs by social stratification. For example, status alone explains less than half of the variance of ideational flexibility (47 per cent); together with status inconsistency, it explains 54 per cent of the variance. The effects of status inconsistency remain statistically significant when respondent's age and his father's occupation -- two variables that are closely related to psychological functioning -- are controlled. The effects of status inconsistency on trustfulness and personally responsible standards of morality even increase slightly in magnitude when the respondent's age and his father's occupation are statistically controlled.

Slomczynski intends now to replicate the analysis with our U.S. data.

THE JAPANESE REPLICATION

Another major replication of the Kohn-Schooler occupations study has been conducted in Japan by Atsushi Naoi and Ken'ichi Tominaga of the Department of Sociology of Tokyo University. Data-collection took place during the summer and fall of 1979. At that time, a probability sample of more than 800 employed men was interviewed, using a questionnaire that asked about job conditions and aspects of psychological functioning in ways comparable to those of the 1964 U.S. study. Data-analysis began in October, 1980, when Naoi came to the Laboratory as a Visiting Scientist to work collaboratively with Carmi Schooler. As reported in previous Annual Reports, Naoi and Schooler developed confirmatory factor-analytic

measurement models of occupational self-direction, intellectual flexibility, and several facets of self-conception and social orientation. These models proved to be generally similar to those that had previously been developed for the American sample. Causal analyses of these data generally confirmed the U.S. findings.

This year, Schooler and Tominaga began an investigation of the social structural determinants of self-directed values in Japan. Using linear structural-equations analysis, they developed a measure of self-directedness of Japanese parental values that corresponds to the measures developed earlier for U.S. and Polish parents. It proved more difficult to develop an appropriate measure of the self-directedness of individuals' values for themselves. Such a measure was eventually developed, but it differs somewhat from the U.S. measure of self-values, in that it includes getting along with others, while the U.S. measure is restricted to conformity to external authority.

Schooler and Tominaga next examined the relative effects on the self-directedness of parental and self-values of several social structural characteristics: occupational status, economic status, educational status, social status of family of origin, age, urbanness, and employment in a relatively traditional versus a more modern sector of the economy. For self-values, occupational and educational status proved by far the most potent determinants, although being younger and working in a non-traditional setting also have statistically significant effects. The powerful effects of occupational and educational status on self-directed values replicate U.S. findings about the importance of social stratification position for the development of self-directed values for oneself.

Social stratificational position as evidenced by occupational and educational status is also among the most important determinants of self-directedness of parental values. Other social-structural variables, though, have relatively greater effects for parental values than for self-values. Thus, age of parent has a stronger effect on parental values than has educational and occupational status, younger fathers having more distinctly self-directed parental values than older fathers. An urban background and a relatively non-traditional work setting also have greater effects on self-directedness of parental- than of self-values. Despite the differences in the relative importance of particular social structural characteristics for parental- and for self-values, the results are congruent with earlier U.S. and Japanese findings about the ways that complex, multifaceted environments affect psychological functioning.

Schooler carried out further analyses to see whether, in Japan as in the U.S. and Poland, the greater degree of occupational self-direction of those in higher social stratification positions is largely responsible for the relationship between social stratificational position and self-directed

values. Despite extensive efforts, however, he has thus far not been able to develop robust, non-anomalous models that test this hypothesis.

During this year, Schooler also began analyses to explore the possibility that the sector of the economy in which an individual works may affect his psychological functioning or may affect the ways that job conditions affect psychological functioning. In the past decade or so, there has been an increased interest in the economic organization of industry, resulting in an extensive literature about a "dual economy." Although there are shades of difference among them, most dual economy theories distinguish between a primary sector, composed of industries marked by high levels of oligopolistic control, large economic scale (including size of work force and assets), relatively large internal labor markets, and relatively secure workers, and a secondary sector, made up of industries in which firms tend to be smaller, both in assets and size, and less well coordinated with each other, and in which workers are generally less well off and less secure. If this distinction is valid, the psychological functioning of individuals may well be affected by the social atmosphere of the sectors in which they work. Thus, working in the secondary sector, where jobs are generally less secure and workers are more subject to arbitrary decisions by superiors, may affect the level of the individual's distress, self-directedness of orientation and intellectual flexibility. Alternatively, job conditions may have different psychological effects on workers in the primary and secondary sectors of the economy.

Some empirical tests have been made of the dual economy conception. The most comprehensive, carried out by Tolbert, Horan, and Beck, used principal component analysis of company characteristics (e.g., assets, profit, number of workers, average weekly wage) in a number of industries to see whether these characteristics covary in the ways that dual economy theory predicts. These investigators extracted a single factor that they see as separating industries into primary and secondary sectors. Schooler has recoded pertinent data from our occupations study about the organizations for which the respondents worked, to take advantage of the coding scheme Tolbert et al. have provided. In addition, in collaboration with Ronald Schoenberg, he is using confirmatory factor analysis to reanalyze the original data on which Tolbert et al.'s analysis was based. Initial analyses suggest that the covariance of company characteristics is best explained by two factors, one factor essentially measuring the company's success in attaining its corporate goals, the second the success of workers in achieving good wages and job security. If further analyses prove that such a factorial solution is appropriate, the psychological effects of these two characteristics of industries will be explored.

Significance of the research:

This research is significant to the mission of the Institute on three distinct levels: (1) It has been well established that the incidence of schizophrenia is inversely related to social-stratification position. This relationship is not simply a function of greater genetic vulnerability of people at lower stratification levels or of more stressful life conditions at those levels. In larger part, it seems to result from people at lower stratification levels having less effective psychological mechanisms for coping with stress and uncertainty. This research, at a very basic level, is investigating what there is about the conditions of life associated with social-stratification position that results in people of lower social-stratification position having less effective mechanisms for coping with stress and uncertainty. (2) Above and beyond NIMH's interest in mental disorder, per se, the Institute has a mandate to study the conditions that facilitate and those that interfere with effective psychological functioning. This research has demonstrated that job conditions have appreciable effects on cognitive functioning, values, self-conceptions, and orientations to the outside world. Much of the work in this research project has focused on (a) demonstrating that job conditions actually do have a causal impact on effectiveness of psychological functioning and (b) elucidating the processes by which job affects psychological functioning. (3) As the research focuses more and more on the effects of social structure on the personality development of children, the potential relevance of what is learned for programs of prevention of mental disorder increases all the more, if only because some of the causal variables are particularly amenable to planned intervention.

Proposed course of further research:

The analysis of the processes by which parents' values and practices affect the values and personality development of their children requires much further analysis, both with respect to the relationship between social stratification and the intergenerational transmission of values and with respect to intrafamily dynamics and the personality development of children. We expect Dr. Jonathan Kelley of the Australian National University to bring to the Laboratory a body of Australian data that will enable us to expand the comparative study of value-transmission both in terms of the number of countries involved in the cross-national analysis and in terms of the range of values and orientations examined. We also intend, after completing the analysis of educational experience and children's personality development, to expand the interpretive model to include education, social stratification, and intrafamily dynamics in a single, comprehensive model of socialization. Finally, as is evident above, the analysis of the Japanese replication is incomplete, with much more to be done, particularly with respect to the comparative analysis of

sector of the economy. Our Japanese collaborators have also collected new data, a survey of the wives of the men in their original survey, which will provide the basis for further comparative study of the relationship between women's job conditions and psychological functioning.

Publications:

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- Slomczynski, M., Janicka, K., and Kacprowicz, G.: Zlozonosc Pracy Jako Zmienna Socjologiczna: Modele Pomiaru i Ich Ocena. (Complexity of Work as a Sociological Variable: Measurement Models and their Evaluation.) Studia Socjologiczne, 3(90): 5-33, 1983.
- Miller, K.A.: The Effects of Industrialization on Men's Attitudes Toward the Extended Family and Women's Rights: A Cross-National Study. J. Marriage Fam. 46(1): 153-160, 1984.
- Schooler, C., Miller, J., Miller, K.A., and Richtand, C.N.: Work for the the Household: Its Nature and Consequences for Husbands and Wives. Am. J. Sociol., July 1984. (In press)
- Kohn, M.L., and Schooler, C.: Praca A Osobowosc: Studium Wspokzaleznosci. (Work and Personality: Study of their Interrelationships.) Warsaw: Polish Scientific Publishers. (In press)
- Kohn, M.L., and Schoenbach, C.: Social Stratification and Parental Values: A Multi-national Assessment. Proceedings of the U.S.-Japan Conference on Social Stratification and Mobility. (In press)
- Kohn, M. L. and Schooler, C.: Shigoto to Personality. (Work and Personality.) Tokyo, Japan: Saiensu-sha Publishing Company. (In press)
- Krauze, T., and Slomczynski, K.: How Far to Meritocracy? Empirical Tests of a Controversial Thesis. Social Forces, 1984. (In press)
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- Naoi, Atsushi, and Schooler, C.: Occupational Conditions and Psychological Functioning in Japan. Am. J. Sociol. (In press)

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Schoenbach, C.: Effects of Husband's and Wife's Social Status on Psychological Functioning. J. Marriage Fam. (In press)

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01.MH 00679-04 LSES

PERIOD COVERED

October 1, 1983, to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structural Equation Models in the Analysis of Data with Measurement Error

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

Ronald J. Schoenberg, Research Sociologist, LSES, NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Socio-environmental Studies

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS

1

PROFESSIONAL:

1

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this work is to further develop the methods and techniques for the specification and estimation of the parameters of structural equation models of survey data that contain random and nonrandom measurement error. Included in this are methods for the identification of the models, estimation of the means of unobserved variables, the determination of model condition, and the treatment of polytomous variables.

Project Description:

A large part of the research conducted by members of this Laboratory depends on a data analysis technique and associated computer program developed and maintained by Ronald Schoenberg. Many revisions and additions were made to the computer program during the year to meet changing demands and to keep the methods used in the Laboratory as current as possible. For example, improved methods were developed and added to the program to aid in the evaluation of the fit of the models. A Generalized Fit Index is now calculated that is not influenced by sample size, and summary measures of explained variance for the structural and measurement models are now computed.

Significant progress has also been made in the analysis of the interaction effects of latent variables. Important problems in this method of analysis have been solved during the year. The greatest flexibility in this kind of analysis will be achieved through the use of factor scores constructed from indicators of the concepts in the model. Interaction effects are studied by multiplying the factor scores together and including these product variables in the model. Factor scores are themselves fallible measures of the true scores and therefore a method for correcting the fallibility had to be developed. In addition, a method was developed to calculate the standard errors of the coefficients of the interaction terms so that correct inferences might be drawn with regard to their statistical significance.

In addition to personal consultation Schoenberg has kept the Laboratory up-to-date on research methods through a weekly one and a half hour seminar. Among the topics covered were methods to handle censored samples, that is, samples in which the dependent variables are restricted in some way; ridge regression, which is a method for the analysis of variables which are so highly inter-correlated that conventional methods become useless; and a method to constrain coefficients in models to certain ranges such as positive values.

Significance of the Research:

Survey data inherently contain a large amount of uncontrolled variation and therefore require sophisticated statistical methods. Because the investigator has little control over the behavior and circumstances of the subjects, and in fact is merely measuring them, then the causal structure among the variables is inherently very complicated. In addition the measurement of the variables nearly always involves some degree of error. Both of these factors dictate the use of sophisticated statistical methods that allow the investigator to specify very complicated causal

structures, as well as simultaneously to control for measurement error. These and other techniques developed in this project are crucial to the conduct of research in the Laboratory.

Proposed Course of Further Research:

In the coming year the methods developed in the project will be extended to the analysis of clinical data. Consultation, classroom training, and statistical research will be provided for investigators in other Laboratories in the Intramural Research Program. Work will continue on the analysis of interaction effects, and on the analysis of categorical variables as well.

Publications:

Schoenberg, R.J. and C. Richtand: An Application of the EM method to the Maximum Likelihood Estimation of Multiple Indicator and Factor Analysis Models. Sociological Research and Methods, May, 1984.

Schoenberg, R.J.: Statistical Models Must Be Appropriate: A Reply to Pat McGowan. Review, April, 1984.

Schoenberg, R.J.: Latent Variables in the Analysis of Limited Dependent Variables, Sociological Methodology . (In press)

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00680-02 LSES

PERIOD COVERED

October 1, 1983, to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Work Experiences and the Deinstitutionalized Mentally Ill

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Elliot Liebow, Guest Researcher, LSES, NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Socio-environmental Studies

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

1

PROFESSIONAL:

1

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The objective of this exploratory, participant observation study is to examine the work experiences of the deinstitutionalized mentally ill over time and to seek out ways in which job characteristics, symptoms, and social relationships interact with one another to effect the course of recovery from psychiatric disorder and reintegration into the community. Field work is being carried out with residents of halfway houses, participants in community-based psychosocial and transitional work programs, and with "unattached" deinstitutionalized men and women. Data collection began in March 3, 1983, and is expected to end on September 30, 1984.

Project Description:

One year ago, Elliot Liebow, on detail to the Laboratory from the Extramural Program, began an exploratory, participant-observer study of the relationship between work experience and recovery from mental illness. The goal of this exploratory research is not to test hypotheses but rather to grasp, so far as possible, the dynamics of the interaction between work experiences and recovery from mental illness.

In this first year, data collection has been based mainly on direct observation and personal interaction with men and women coming out of Springfield State Hospital and following them through their reentry into their home communities, focussing particularly on the role of work experiences in this process. The study population is drawn mainly from half-way houses, psychosocial and vocational programs, soup kitchens and emergency shelters in Montgomery County, Md.

Significance of the research:

This project is directly pertinent to our understanding of rehabilitation of the deinstitutionalized mentally ill.

Proposed course of further research:

The data collection phase is expected to end in September of this year. For the remaining five months, the project will continue to focus sharply on the individual work experiences over time of deinstitutionalized persons, but a greater effort will be made to place these experiences in a larger social and mental health system context. The social context will be further developed through direct personal contacts, formal and informal, with employers, supervisors, co-workers, friends, parents and other relatives. The mental health system context will be constructed from interviews with hospital administrators in charge of vocational programs and discharge planning, after-care teams, case managers, and Federal, state and county representatives of vocational rehabilitation programs and other relevant social and rehabilitative mental health services.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00424-09 LCB

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biologically Active Peptides in the Brain

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Michael J. Brownstein, Chief, Laboratory of Cell Biology, NIMH

see attached

COOPERATING UNITS (if any)

Stanford U. School of Med.,
LNN/CH, Johns Hopkins, LCS/NIMH, LMG/CH, CH/NIADDK

LAB/BRANCH

Laboratory of Cell Biology

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

10

PROFESSIONAL:

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have continued to study the distribution of peptide-containing cells in the central nervous system, the biosynthesis of biologically active peptides, and the factors that regulate peptide secretion. Our studies of a number of peptides have contributed to a better understanding of the cell biology of peptidergic neurons and of their role in the brain.

Other Professional Personnel Engaged on Project

L. Eiden	Sr. Staff Fellow	LCB, NIMH
M. Palkovits	Visiting Scientist	LCB, NIMH
R. Pruss	Sr. Staff Fellow	LCB, NIMH
E. Mezey	Visiting Associate	LCB, NIMH
J. Kiss	Visiting Associate	LCB, NIMH
F. Antoni	Visiting Associate	LCB, NIMH
V. Hook	Guest Researcher	LCB, NIMH
N. Zamir	Visiting Fellow	LCB, NIMH
J. Russell	Sr. Staff Fellow	LCB, NIMH
J. Moskal	Sr. Staff Fellow	LCB, NIMH
T. Bonner	Res. Biophysicist	LCB, NIMH
M. Bannon	Guest Researcher	LCB, NIMH
H. Affolter	Guest Researcher	LCB, NIMH
P. Giraud	Guest Researcher	LCB, NIMH
C. Hsu	Phys. Sci. Tech.	LCB, NIMH
A. Iacangelo	Microbiologist	LCB, NIMH
R. Siegel	Staff Fellow	LCB, NIMH
A. Namboodiri	Research Associate	LNN, CH
H. Gainer	Chief	LNN, CH
P. Loh	Section Chief	LNN, CH
H. Okayama	Visiting Scientist	LMG, CH
A. Hotchkiss	Sr. Staff Fellow	CH, NIADDK
E. Weber	Associate Professor	Stanford U. School Med.
L. Fricker	Postdoc. Fellow	Johns Hopkins
S. Snyder	Professor	Johns Hopkins
T. Reisine	Sr. Staff Fellow	LCS, NIMH
J. Axelrod	Guest Researcher	LCS, NIMH

Project Description

Drs. Siegel, Brownstein, and Okayama are attempting to use expression cloning in mammalian cell lines to characterize neurotransmitter receptor mRNA's. This long term project should result in a better understanding of the structure and function of receptors.

Drs. Brownstein, Gainer and Loh are continuing to investigate mechanisms of peptide precursor processing. Work in progress indicates that processing is more complex than heretofore suspected. In addition to a trypsin-like endopeptidase that recognizes pairs of bases and a carboxypeptidase B-like enzyme, a second endopeptidase that cleaves after single bases and an aminopeptidase that trims off basic residues from the N-terminus of peptides may be involved in processing.

Dr. Hook has studied one of the above enzymes in detail: the carboxypeptidase B-like enzyme named carboxypeptidase E to distinguish it from the former. She has developed specific antibodies against it (with Drs. Fricker and Snyder) and in

collaboration with Drs. Mezey, Pruss, and Siegel, has used these for immunocytochemistry. She has also developed a RIA for the enzyme and should now be able to study the regulation of its synthesis. She has developed an assay for the trypsin-like enzyme that cleaves between or after pairs of bases and has begun to isolate and characterize it.

Drs. Zamir, Palkovits, Mezey, Weber, and Brownstein have shown that destruction of fibers traveling from the basal ganglia to the substantia nigra results in a marked decline in dynorphin-related but not met-enkephalin-related peptides. The lesions also cause leu-enkephalin levels in the substantia nigra to fall precipitously. Thus, it seems that leu-enkephalin can be liberated from the dynorphin/neuroendorphin precursor.

Drs. Namboodiri and Brownstein have studied the distribution of an enzyme that acetylates aspartic (but not glutamic) acid. This enzyme may be involved in the biosynthesis of N-acetyl Asp Glu.

Drs. Mezey, Reisine, Palkovits, and Axelrod have continued to study the role of peripheral catecholamines in regulating pituitary ACTH secretion. They have shown that catecholamines mediate the outpouring of ACTH that is seen after insulin administration.

Drs. Mezey, Kiss, Reisine, and Axelrod have shown that the CRF-producing neurons in the paraventricular nucleus receive a dense adrenergic innervation and that blockade of adrenaline synthesis with PNMT inhibitors causes a significant increase in CRF production.

Dr. Kiss and Dr. Mezey have found that the same set of cells that make CRF can also synthesize vasopressin and cholecystokinin. This is especially apparent in adrenalectomized animals. Together with Dr. Reisine they have shown that vasopressin plus CCK are as effective as CRF in releasing ACTH from cultured pituitary cells.

Dr. Moskal has prepared and identified a monoclonal antibody that appears to bind to a molecule(s) involved in modulating a specific glycoprotein sialyltransferase since its removal from solubilized Golgi preparations by immunoprecipitation results in a marked elevation in this enzyme activity. He has also found a four-fold increase in the specific glycoprotein sialyltransferase activity mentioned above when NG 108-15 cells are differentiated with dibutyryl-cyclic AMP. Retinoic acid, on the other hand, elevates another sialyltransferase--one involved in the biosynthesis of GM3 ganglioside. Finally, when NG 108-15 cells are differentiated with dibutyryl-cyclic AMP and cocultured with primary muscle cultures the activity of the galactosyltransferase involved in production of lactoneotetraosylceramide was

increased. Dr. Moskal is attempting to identify the factors that cause these changes in enzyme activity.

Dr. Moskal has raised monoclonal antibodies to five day postnatal rat dentate gyrus granule cells. The antibodies recognize cell surface antigens and have proven useful for fluorescence activated cell sorting. Thus normal and abnormal (reeler mutant) granule cells can be separated and their surface molecules compared.

Dr. Russell has studied two transport processes in neurosecretory vesicles: 1) the transport of protons and 2) the transport of electrons. The first of these is mediated by an ATP-dependent system and functions to maintain the intravesicular pH between 5.2 and 5.7. The low intragranular pH may promote condensation of peptide precursors and aggregation of products. It may also be optimal for (or regulate) the activity of processing enzymes. The electron transfer mediator appears to be a cytochrome (b₅₆₁) in the granule wall. Transport of electrons to the inside of the granules is essential for the activity of the peptide amidating enzyme.

Dr. Pruss has raised a monoclonal antibody against bovine cytochrome b₅₆₁, has used it to examine the tissue distribution of the cytochrome, and plans to examine the regulation of its production.

Drs. Pruss, Siegel, and Eiden have studied the effects of drugs on peptide levels in cultured chromaffin cells. VIP levels increase markedly in the cultured cells; the mechanism of this effect is being sought.

Drs. Eiden, Affolter, Giraud, Hotchkiss, Siegel, and Pruss have investigated the effects of nicotine, forskolin, potassium, and reserpine on enkephalin and related peptides and on messenger RNA coding for proenkephalin (mRNA^{enk}). Nicotine and forskolin increase intracellular enkephalin and mRNA^{enk} levels, nicotine rapidly and forskolin more slowly. Depolarizing agents (e.g., K⁺) also increase enkephalin and mRNA^{enk} at the same time that they provoke release. It appears that stimulation of enkephalin biosynthesis occurs via enhanced enkephalin gene transcription following an increase in intracellular cyclic AMP, and may depend on calcium influx as well. Bovine enkephalin gene has been isolated and studies are in progress of transcriptional regulation of mRNA^{enk} production.

Dr. Eiden, Ms. Iacangelo, and Ms. Hsu have established a RIA for chromogranin A and have shown that this molecule constitutes about 10 percent of the total protein in chromaffin cells. They have prepared a bovine adrenal medulla cDNA library and are screening this library for chromogranin A clones.

Drs. Bannon, Giraud, Affolter, and Eiden have developed a method for quantitating mRNA^{enk} in discrete brain regions and hope to use it to gauge neuropeptide turnover. They have shown that haloperidol increases mRNA^{enk} within several hours (at a time when enkephalin concentrations are unchanged).

Dr. Giraud has used pulse-chase methodology to show that drugs which increase or mimic cyclic AMP increase enkephalin precursor and enkephalin pentapeptide production by adrenal medullary cells. Cholinergic agents have the same effect while reserpine acts posttranslationally to stimulate the processing of enkephalin precursor as opposed to its synthesis.

Drs. Siegel and Kiss have made significant progress towards developing a reliable and well validated method for localizing (and quantitating) mRNA by in situ hybridization cytochemistry. They have found that most cultured chromaffin cells have mRNA^{enk} and that following growth in depolarizing medium message levels increase. Preliminary attempts to combine in situ hybridization and immunocytochemistry have been encouraging.

Dr. Antoni has found that the anterior pituitary has a single class of high affinity binding sites for arginine vasopressin and that these sites are different from those previously described in rat tissues. The adrenal medulla also has vasopressin binding sites identical to those in bovine liver membranes. The role of vasopressin in the adrenal medulla is under investigation.

Significance to Biomedical Research

Nerve cells use chemical "transmitters" to communicate with one another and with other target cells. Changes in transmitter biosynthesis, release, and/or metabolism have been suggested to result in nervous and mental disorders. Death of dopaminergic neurons in the substantia nigra, for example, is associated with the symptoms of Parkinson's disease. In the last ten years the number of putative neurotransmitters has increased by a factor of four or five. Most of the newly detected chemical messengers are peptides. Our knowledge of the anatomy, physiology and pharmacology of peptidergic neurons is comparatively incomplete at present; indeed, it is clear that many biologically active peptides remain to be isolated and characterized. The work outlined above is principally devoted to improving our understanding of cells. To the extent that we understand these cells, we can formulate better hypotheses about their role in causing disease.

Proposed Course

The work outlined above is still in progress and will be continued.

Publications

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Zamir, N., Palkovits, M., and Brownstein, M.J.: Distribution of immunoreactive dynorphin in the central nervous system of the rat. Brain Res. 280: 81-93, 1983.

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Mezey, E., Reisine, T.D., Palkovits, M., Brownstein, M.J., and Axelrod, J.: Direct-stimulation of β 2-adrenergic receptors in rat anterior pituitary induces the release of adrenocorticotropin in vivo. Proc. Natl. Acad. Sci. USA 80: 6728-6731, 1983.

Eiden, L.E. and Hotchkiss, A.J.: Cyclic adenosine monophosphate regulates vasoactive intestinal polypeptide and enkephalin biosynthesis in cultured bovine chromaffin cells. Neuropeptides 4: 1-9, 1983.

Handelmann, G.E., Russell, J.T., Gainer, H., Zerbe, R., and Bayorh, M.: Vasopressin administration to neonatal rats reduces antidiuretic response in adult kidneys. Peptides 4: 827-832, 1983.

Zamir, N., Palkovits, M., Weber, E., Mezey, E., and Brownstein, M.J.: A dynorphinergic pathway of leu-enkephalin production in rat substantia nigra. Nature 307: 643-645, 1984.

Mezey, E., Leranth, C., Brownstein, M.J., Friedman, E., Krieger, D.T., and Palkovits, M.: On the origin of the serotonergic input to the intermediate lobe of the rat pituitary. Brain Res. 294: 231-237, 1984.

Ruth, J.A. and Eiden, L.E.: Leucine-enkephalin modulation of catecholamine positive chronotropy in rat atria is receptor-specific and calcium-dependent. Neuropeptides 4: 101-108, 1984.

Hook, V.Y.H.: Carboxypeptidase B-like activity for the processing of enkephalin precursors in the membrane component of

bovine adrenomedullary chromaffin granules. Neuropeptides 4: 117-126, 1984.

Kiss, J.Z., Mezey, E., and Skirboll, L.: Corticotropin-releasing factor-immunoreactive neurons of the paraventricular nucleus become vasopressin positive after adrenalectomy. Proc. Natl. Acad. Sci. USA 81: 1854-1858, 1984.

Palkovits, M., Kiss, J.Z., Beinfeld, M.C., and Brownstein, M.J.: Cholecystokinin in the hypothalamo-hypophyseal system. Brain Res. 299 186-189, 1984.

Zamir, N., Palkovits, M., Weber, E., and Brownstein, M.J.: Distribution of immunoreactive dynorphin B in discrete areas of the rat brain and spinal cord. Brain Res. 300: 121-127, 1984.

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Antoni, F.: Characterization of high affinity binding sites for vasopressin in bovine adrenal medulla. Neuropeptides (in press), 1984.

Palkovits, M., Brownstein, M.J., and Zamir, N.: On the origin of dynorphin A and α -neo-endorphin in the substantia nigra. Neuropeptides (in press), 1984.

Zamir, N., Palkovits, M., and Brownstein, M.J.: Distribution of immunoreactive dynorphin A(1-8) in discrete nuclei of the rat brain: Comparison with dynorphin A. Brain Res. (in press), 1984.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00422-13 LCB

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neuropharmacology of Circadian Rhythms

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	M. Zatz	Med. Officer (Res.)	LCB, NIMH
Others:	J. Moskal	Sr. Staff Fellow	LCB, NIMH
	R. Siegel	Staff Fellow	LCB, NIMH
	E. Mezey	Visiting Associate	LCB, NIMH
	L. Eiden	Sr. Staff Fellow	LCB, NIMH
	S. Reppert	Asst. Professor	Massachusetts General Hospital
	L. Tamarkin	Sr. Staff Fellow	DEB, NICHD
	L. Skirboll	Sr. Staff Fellow	BPB, NIMH

COOPERATING UNITS (if any)

Department of Pediatrics, Massachusetts General Hospital, Boston, Massachusetts, DEB/NICHD, BPB/NIMH

LAB/BRANCH

Laboratory of Cell Biology

SECTION

Unit on Biochemical Pharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

1.0

PROFESSIONAL:

0.9

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Circadian rhythms and environmental lighting regulate a number of endocrine and behavioral functions. The suprachiasmatic nucleus of the hypothalamus appears to be the circadian pacemaker in mammals. The chick pineal gland remains rhythmic and responsive to light in vitro. Attempts are underway to generate a monoclonal cell line of chick pineal cells that will maintain circadian rhythmicity indefinitely in culture.

Project Description

Objectives: To elucidate the biochemical mechanisms and neuropharmacology of circadian rhythms.

Methods: Biochemical, pharmacologic, surgical, cell culture, radioimmunologic, and radioenzymatic techniques.

Major Findings: There is strong but indirect evidence that mammalian circadian rhythms are endogenously generated in the suprachiasmatic nucleus (SCN) of the hypothalamus. Cells in this nucleus contain a number of neurotransmitters, including VIP and vasopressin. Neonatal rat SCN cells were dispersed and maintained in culture for up to five weeks in attempts to develop a system in which the regulation of SCN rhythmicity could be studied in vitro. However, no measurable VIP or vasopressin was detected at any time, in the medium or in the cells. The project has been discontinued.

The paraventricular nucleus (PVN) of the hypothalamus has been implicated in transmitting the circadian output of the SCN to the rat pineal. Attempts to demonstrate increased pineal activity consequent to electrical stimulation of the PVN (using several stimulus parameters and durations) failed. The project has been discontinued.

The chick pineal is capable of maintaining a circadian rhythm of melatonin secretion in culture for several days. We are attempting to generate an immortal cell line from chick pineal cells which, ideally, would maintain light sensitivity and circadian rhythmicity in culture indefinitely. Using a temperature-sensitive mutant of Rous sarcoma virus we have succeeded in transforming pineal cells from hatchling chicks and maintaining them in culture for 16 weeks. Cell morphology and growth rates were affected, as hoped, with changes in temperature. Melatonin production was maintained but fell off with long term culture.

Significance to Biomedical Research: Circadian rhythms occur in hormone levels, activity, mood, temperature, and other physiologic functions. Elucidation of the mechanisms generating and regulating circadian rhythms are of broad clinical and biologic interest.

Proposed Course of Project: Clones of transformed chick pineal cells will be screened for their ability to secrete melatonin, respond to light and other relevant agents, and maintain circadian rhythmicity. Clones with these properties would permit in depth analysis of the biochemical mechanisms generating and regulating circadian rhythms.

Publications

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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00429-05 LCB

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biochemistry of Membranes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: M. Zatz Med. Officer (Res.) LCB, NIMH

Other: P. J. O'Brien Section Chief LVR, NEI
T. Reisine Sr. Staff Fellow LCS, NIMH

COOPERATING UNITS (if any)

LVR/NEI, LCS, NIMH

LAB/BRANCH

Laboratory of Cell Biology

SECTION

Unit on Biochemical Pharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

2.0

PROFESSIONAL:

1.6

OTHER:

0.4

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

- a) The enzyme which transfers long chain fatty acids from acyl coenzyme A to rhodopsin has been solubilized and characterized. This newly discovered enzyme mediates a new class of posttranslational modification of membrane proteins, including receptors.
- b) A method has been developed to assess inositide metabolism in small amounts of tissue. In the rat pineal gland, the stimulatory effect of beta-adrenergic agents are potentiated by alpha-adrenergic stimulation. Lithium, at therapeutic doses, potentiates the effect of alpha stimulation, while having no effect on its own. Inositide metabolism is concomitantly affected. Phorbol esters mimic the effect of alpha-adrenergic stimulation.
- c) Lithium stimulates ACTH secretion by anterior pituitary cells in culture and by pituitary tumor (AtT-20) cells. Inositide metabolism is concomitantly affected. Lithium desensitizes the cells to subsequent stimulation by lithium or by phorbol esters, but not by other agonists.

Project Description

Objectives: a) To elucidate the nature and function of protein acylation. b) To determine the relationship between alpha-adrenergic stimulation, inositide metabolism, and the effects of lithium. c) To determine the relationship between inositide metabolism, phorbol esters, and the effects of lithium on ACTH secretion.

Methods: Biochemical, chromatographic, chemical, pharmacologic, tissue culture, and radioactive trace techniques.

Major Findings: a) Recent reports indicate that long chain acyl groups are covalently attached to a number of intrinsic membrane glycoproteins. All previous studies have used radioactive fatty acid and whole cells. We previously demonstrated the incorporation of ³H-palmitate into rhodopsin, the intrinsic membrane glycoprotein which mediates photoreception. We have now succeeded in demonstrating the specific transfer of palmitate from palmityl coenzyme A to rhodopsin using partially purified rod outer segments. The enzyme and substrate have been solubilized, permitting partial characterization of the reaction. b) There has been a recent resurgence of interest in the relationships between inositide metabolism and receptor function. A number of receptors--those intimately involved with calcium fluxes--strongly affect inositide turnover. A method has been developed, using ³H-inositol, Sephadex, and Dowex columns to separate virtually all the inositides and assay them individually in small amounts of tissue. This method has been applied to rat pineal and to cultured cells. In the rat pineal, inositide turnover is stimulated by alpha-adrenergic stimulation. This effect can be seen as early as one minute after the addition of norepinephrine. There is a marked increase in the response after denervation. The physiologic effect of alpha stimulation is potentiation of beta-adrenergic stimulation of serotonin N-acetyltransferase (SNAT) induction. Phorbol esters, which stimulate protein kinase C, mimic the effect of alpha stimulation. Lithium, which is used therapeutically in manic-depressive disease, potentiates the action of alpha-adrenergic stimulation or of phorbol ester. However, it had no effect in the absence of these agents. Lithium is known to prevent the cleavage of inositol phosphate to inositol, but this cannot account for the effect described. Thus, lithium acts in this system to potentiate physiologic potentiation, without either stimulating or potentiating on its own. c) Using the methods developed for the pineal, we examined the effects of various agents on inositide metabolism in AtT-20 cells, a pituitary tumor cell line which responds to a number of agents by secreting ACTH. Surprisingly, lithium itself also stimulated ACTH secretion. Its effects on secretion and on inositol phosphate remained correlated under several conditions. Lithium inhibited

the effect of CRF somewhat. Again, these effects of lithium cannot readily be explained by current models of its mechanisms of action. ACTH secretion caused by lithium is calcium dependent and inhibited by somatostatin or dexamethasone. Lithium pretreatment desensitizes the cells to subsequent stimulation by lithium, while the response to potassium or CRF remains unaffected. Phorbol esters also stimulate ACTH secretion, and are thought to act distal to the inositide system. However, lithium pretreatment also desensitizes the cells to subsequent stimulation by phorbol ester. Thus, in this system, lithium acts as an agonist, comparable to CRF in effectiveness, to stimulate and desensitize the cells.

Significance to Biomedical Research: a) Posttranslational modifications of receptors provide a mechanism for the regulation of the actions of drugs, hormones, and neurotransmitters. b) Alpha-adrenergic receptors are part of a class of receptors for neurotransmitters and hormones that act through calcium and affect inositide metabolism. Elucidation of the mechanisms transducing their stimulation into physiologic effects is important to understanding how neurotransmitters work and to finding out where and how lithium works. c) A stimulatory effect of lithium, at therapeutic concentrations, is unusual. Elucidation of its mechanism of action may shed light on the therapeutic action of lithium as well as on the regulation of ACTH secretion (ACTH is itself an important hormone).

Proposed Course of Project: a) Protein acyltransferase will be separated from its substrate. Factors regulating its activity (such as light) will be tested. Consequences of its activity on the physiology of rhodopsin will be sought. Acylation of other receptors and regulatory proteins will be explored. b) Interactions between beta-adrenergic stimulation, alpha-adrenergic stimulation, lithium, inositide metabolism, and phorbol esters will be characterized. The mechanism of action of lithium, possibly involving protein kinase C, will be sought. c) Interactions between lithium, phorbol esters, ACTH secretion, and inositide metabolism will be characterized. Lithium's effects on inositide turnover and protein kinase C activity will be investigated.

Publications:

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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00427-07 LCB

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

On the Mechanism of Signal Transduction Through Receptors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Fusao Hirata, Visiting Scientist, Laboratory of Cell Biology, NIMH

see attached

COOPERATING UNITS (if any)

LBG/NHLBI, LDBA/NIDR, LID/NCI, Johns Hopkins University

LAB/BRANCH

Laboratory of Cell Biology

SECTION

Unit on Biochemistry

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

5.0

PROFESSIONAL:

5.0

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Glucocorticoids exert a variety of actions on tissues and organs in the body. Anti-inflammatory activity, a major action of these hormones, has been shown to be associated with induction of the synthesis of phospholipase inhibitory protein, lipomodulin, in target tissues. Purified lipomodulin mimics most, if not all, of glucocorticoid actions such as anti-inflammation, immunosuppression, arrest of cell growth and promotion of cellular differentiation. All these actions have been attributed to inhibition of phospholipases in plasma membranes, which in turn, alters permeability and intracellular pH. Such changes in intracellular ion compartmentation are suggested to play an important role in the blockade of signal transduction by glucocorticoids.

Other Professional Personnel Engaged on Project

Y. Notsu	Guest Researcher	LCB, NIMH
K. Matsuda	Guest Researcher	LCB, NIMH
C. Pezzoli	Guest Researcher	LCB, NIMH
T. Matsumoto	Guest Researcher	LCB, NIMH
T. Hattori	Visiting Fellow	LCB, NIMH
G. Kunos	Guest Researcher	LCB, NIMH
I. Kunos	Guest Researcher	LCB, NIMH
R. Yamada	Guest Researcher	LCB, NIMH
R. Herberman	Chief	LID, NCI
E. Schiffman	Biologist	LDBA, NIDR
M. Nirenberg	Chief	LBG, NHLBI
D. Newcombe	Professor	Johns Hopkins
K. Ishizaka	Professor	Johns Hopkins
S. Katz	Chief	DB, NCI
T. Shimada	Visiting Fellow	DB, NCI

Project Description

Objectives: To study the mechanism of signal transduction including regulation of ion fluxes, cyclic nucleotide production, glucose metabolism and gene expression.

Methods: Enzymatic, biochemical, immunological, pharmacological and molecular biological techniques.

Major Findings: Lipomodulin, a phospholipase inhibitory protein, whose synthesis is induced by glucocorticoids, has been purified nearly to homogeneity from media conditioned with HL 60 cells and from calf and human sera. This purified protein inhibits (1) chemotaxis of neutrophils and monocytes, (2) immunoglobulin synthesis, (3) the cytotoxic reaction, and (4) cell growth. Furthermore, it regulates functions of α_1 and β -adrenoceptors in hepatocytes and of growth factor receptors in lymphocytes. Using HL 60 cells as a model, we have studied the mechanism by which phorbol esters and retinoic acids induce cellular differentiation. The differentiation promoting activities of these compounds can be blocked by amiloride, an inhibitor of the Na^+/H^+ exchanger and ouabain, an inhibitor of Na^+/K^+ -ATPase, suggesting that ion fluxes play an important role in signaling. Phorbol esters enhance phospholipases and retinoic acids suppress them. Lipomodulin also promotes cellular differentiation of neuroblastoma cells and U937 cells by a similar mechanism.

Significance to Biomedical Research: Stress usually causes elevated serum levels of glucocorticoids, which often causes immunosuppression. Since these hormones induce the synthesis of lipomodulin, in a variety of tissues and organs, it is quite likely that lipomodulin is a mediator of some actions of stress hormones.

Glucocorticoids are known to enhance catecholamine turnover. Lipomodulin mimics this action of the steroids, as measured by their effect on the adrenergic expression of NH15CA2 neuroblastoma hybrid cells, or superior cervical ganglion cells. These observations suggest that the development of autonomic nervous system in the fetus will be affected by the stress to his mother.

Proposed Course of Project: The effect of lipomodulin and its degrading enzyme (tentatively designated as lipomodulinase) will be tested on the development of autonomic nervous system, using primary cultures of superior cervical ganglion cells and NH15CA2 cells as model systems.

Recently, we have isolated cDNA clones possibly encoding lipomodulin. We will confirm that these clones are the lipomodulin gene by (1) transfection experiments, (2) sequencing these clones, and (3) isolation of lipomodulin proteins from the transformed E. coli. Such confirmation is necessary to the next steps where the genomic gene of lipomodulin is isolated to find the locating site of "glucocorticoid-enhancer." "Glucocorticoid-enhancer" site is suggested as the site where the glucocorticoid-receptor complex binds to enhance the expression of the gene.

Publications

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Schiffman, E., Geetha, V., Pancev, D., Warabi, H., Mato, J.,

Hirata, F., Brownstein, M., Manjunath, R., Mukgerjee, A., Liotta, S., and Terranova, V.P.: Adherence and regulation of leukotaxis. In Keller, H.U. and Till, G.O. (Eds.): Agents and Actions Supplement. Basel, Birkbauser Verlag, 1983, Vol. 12, pp. 106-120.

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Hirata, F.: Receptor mediated cascade of phospholipid metabolism. In Horrock, L. (Ed.): Phospholipids in Nervous System. New York, Raven Press (in press), 1984.

Hirata, F., Notsu, Y., Matsuda, K., and Hattori, T.: Modulation of neuroreceptor functions by lipomodulin, a phospholipase inhibitory protein. In Olsen, R. (Ed.): Regulation of Neuroreceptors Function. New York, Raven Press (in press), 1984.

Hirata, F., Notsu, Y., Matsuda, K., Vasanthakumar, G., Schiffman, E., Wong T.-W., and Goldberg, A.R.: Inhibition of leucocyte chemotaxis by Glu-Glu-Glu-Glu-Tyr-Pro-Met-Glu and Leu-Ile-Glu-Asp-Asn-Glu-Tyr-Thr-Ala-Arg-Gln-Gly. Biochem. Biophys. Res. Commun. 118: 682-690, 1984.

Namiuchi, S., Kumagai, S., Imura, H., Suginosita, T., Hattori, T., and Hirata, F.: Quinacrine inhibits the primary but not secondary proliferative response of human cytotoxic T cells to allogenic non-T cell antigens. J. Immunol. 132: 1456-1461, 1984.

Hirata, F., Notsu, Y., Matsuda, K., Hattori, T. and DelCarmine, R.: Phosphorylation at tyrosine site of lipomodulin, a phospholipase inhibitory protein, in mitogen-stimulated murine thymocytes. Proc. Natl. Acad. Sci. USA (in press), 1984.

Peters-Golden, M., Hirata, F., and Newcombe, D.: Glucocorticoid inhibition of zymosan-induced arachidonic acid release by rat alveolar macrophages. Am. Rev. Respir. Dis. (in press), 1984.

Hirata, F.: Phospholipid methylation and signal transduction.

In Delgado-Escueta, A.V. (Ed.): International Symposium on Basic Mechanisms of Epilepsy. New York, Raven Press, 1984 (in press).

Hirata, F.: Lipomodulin; a modulator of cellular phospholipid metabolism. In Cheung, W.Y. (Ed.): Calcium and Cell Function. New York, Academic Press, 1984 (in press).

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 01836-06 NS

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Receptors in the Central Nervous System: Biochemistry to Behavior

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: S. M. Paul Chief NS, NIMH

Others: P. Skolnick Pharmacologist LBC, NIADDK
 R. D. Schwartz Pharmacologist NS, NIMH
 R. J. Weber Staff Fellow NS, NIMH
 A. J. Janowsky Staff Fellow NS, NIMH
 J. N. Crawley Res. Biologist NS, NIMH
 D. W. Hommer Staff Psychiatrist NS, NIMH

COOPERATING UNITS (if any)

Laboratory on Bioorganic Chemistry, NIADDK; Clinical Psychobiology Branch, NIMH;
 Section on Brain Biochemistry, NS, NIMH; Clinical Neuropharmacology Branch, NIMH,
 Section on Molecular Pharmacology, NS, NIMH; NICHD, Univ. of Wisconsin

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Section on Preclinical Studies

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

4.0

PROFESSIONAL:

3.5

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

High affinity and stereospecific receptors for benzodiazepines are present in the mammalian central nervous system. It is currently believed that the interaction of benzodiazepines with their receptors initiates a series of neuronal events resulting in an enhancement of GABA-mediated chloride permeability. The latter results behaviorally in the major pharmacological actions of benzodiazepines, namely their anxiolytic, anticonvulsant, hypnotic, and muscle relaxant actions. In addition to benzodiazepines, a variety of sedative/hypnotic agents of the minor tranquilizer class (e.g. the barbiturates) appear to interact with one or more components of the benzodiazepine/GABA receptor complex, and thus the latter has been proposed as a common site of minor tranquilizer action. Several aspects of the benzodiazepine/GABA receptor complex are currently being studied, including purification of the receptor, characterization of multiple binding sites on the receptor complex which recognizes agonists, antagonists or inverse agonists, the development of anti-idiotypic antibodies to the various binding site domains on the complex studies on the behavioral and biochemical effects of novel (non-benzodiazepine) anxiolytics as well as "anxiogenic" inverse agonists, and the identification of a novel benzodiazepine receptor in the CNS and peripheral tissues for 4-chlorodiazepam (Ro5-4864), the so-called peripheral benzodiazepine receptor ligand. Recent work has also focused on developing an *in vitro* system, using a subcellular preparation from rat brain (the synaptoneurosome), for studying barbiturate and GABA-mediated chloride flux and has resulted in the first reliable method for studying chloride flux in brain.

Other Professional Personnel:

W. B. Mendelson	Staff Psychiatrist	CP NIMH
T. R. Insel	Staff Psychiatrist	CN NIMH
E. S. Kempner	Physicist	LPB NIADDK
J. R. Glowa	Senior Staff Fellow	NS NIMH
J. W. Thomas	Chemist	NS NIMH
G. P. Chrousos	Staff Physician	DNB NICHHD
J. M. Cook	Chemist	NICHHD, Univ. of Wisconsin
M. M. Schveri	Staff Fellow	LPB NIADDK
G. T. Bolger	Visiting Fellow	LBC NIADDK
B. A. Weissman	Visiting Scientist	LBC NIADDK
K. H. Weber	Guest Researcher	LBC NIADDK
C. B. Pert	Senior Staff Fellow	NS NIMH

Project Description:Methods Employed:

Radioreceptor techniques have been employed in the neurochemical characterization of benzodiazepine receptors and as a means of detecting and quantifying endogenous substances which may regulate these sites. Other biochemical techniques employed include radioenzymatic assays, thin layer, ion exchange and high pressure liquid chromatography, gel filtration and molecular size exclusion chromatography. A variety of in vitro biochemical assays for measuring anion transport in subcellular brain preparations have been developed and are currently being used. Radiation inactivation has been employed to estimate the functional target size (viz. molecular weights) of various receptor proteins comprising the benzodiazepine/GABA receptor complex. Pharmacologic testing in animals includes quantitation of the sensitivity of mice to chemical convulsants. Ataxia and muscle relaxation have been tested through the use of rotating rod and "wire-grip" procedures. Anxiolytic (anticonflict) action has been examined using either a mouse model of behavior (which measures the activity of mice in a novel environment) or alternatively, using a rat "conflict" model of behavior (the thirsty rat conflict test). Blood pressure in rodents has been measured using an indirect (tail cuff) technique. Anxiolytic/anxiogenic behavior in primates is currently being investigated using behavioral rating scales (modified Redmond) as well as measuring the somatic and endocrine markers of anxiety (pulse rate, mean arterial blood pressure, and plasma catecholamines, ACTH, β -endorphin and cortisol). Recently classical conditioning methods have been established in primates to assess the "punishing" and "rewarding" aspects of anxiogenic and anxiolytic compounds.

Major Findings:

Previous studies from this laboratory have shown that a novel series of "annelated" 1,4-diazepines ("hetrazepines") have high affinities for "brain-type" benzodiazepine receptors in vitro and possess anticonflict and anticonvulsant but not hypnotic activity in vivo. In contrast to classical 1,4-benzodiazepines (e.g. diazepam), the apparent affinities of these compounds are not increased by

γ -aminobutyric acid (GABA) under a variety of experimental conditions. These observations invalidate the proposed *in vitro* procedure reported by this laboratory and others (1982) as a facile *in vitro* assay to discriminate benzodiazepine "agonists" from "antagonists". The electrophysiological actions of several tetrazepines have now been examined in rat substantia nigra zona reticulata, an area of the brain previously reported to be responsive to both benzodiazepines and γ -aminobutyric acid. WE 973 and WE 1008 (two tetrazepines that do not appear to possess hypnotic properties (and do not have their apparent affinities altered by GABA) inhibit the spontaneous firing of these neurons. However, in contrast to "classical" 1,4-benzodiazepines and a hypnotic tetrazepine (brotizolam; WE 941), neither WE 973 nor WE 1008 augment the inhibition of cell firing elicited by iontophoretic application of GABA. These results strongly suggest that direct occupation of benzodiazepine receptors by high affinity ligands may be a sufficient condition to elicit an anticonflict and anticonvulsant action, and that the well described augmentation of GABAergic transmission by classical 1,4-benzodiazepines may be responsible for a hypnotic action.

Previous studies from this laboratory have established that injection of certain C-3 substituted β -carbolines represents a robust, chemically induced model of anxiety in primates. Several compounds which possess anticonflict or anxiolytic actions but do not interact with benzodiazepine receptors *in vitro* have now been examined in this model. The serotonin antagonist cyproheptadine was shown to block some, but not all, of the repertoire of behaviors produced by injection of 3-carboethoxy- β -carboline (β -CCE). However, cyproheptadine was unable to reverse the somatic changes (e.g. increases in heart rate and blood pressure) produced by β -CCE. Although cyproheptadine antagonized β -CCE induced increases in plasma cortisol and ACTH, it was unable to antagonize increases in plasma norepinephrine and epinephrine induced by β -CCE. A non-sedating dose (1 mg/kg, i.v.) of the GABA-mimetic THIP displayed a markedly different profile against β -CCE. Some, but not all of the behaviors produced by β -CCE were antagonized by THIP. THIP was able to antagonize the increase in heart rate produced by β -CCE but not the increase in blood pressure. Further, THIP did not block increases in plasma ACTH, partially antagonized the rise in plasma norepinephrine, but was unable to antagonize the rise in plasma epinephrine produced by β -CCE. Thus, studies with a number of "atypical" anxiolytic or anticonflict agents including propranolol, clonidine, cyproheptadine, and THIP suggest that an anxiolytic actions may be produced through activation of several neurochemical pathways or through a peripheral mechanism (e.g. with propranolol) bypassing the benzodiazepine-GABA receptor-chloride ionophore complex.

Numerous clinical studies have demonstrated an association between the development of depressive states and anxiety. Since administration of β -carbolines appears to be a chemically induced model of anxiety, we examined the ability of an anxiogenic β -carboline (FG 7142) to induce "learned helplessness" behavior (thought to be an animal model of depression) in rodents. A single injection of FG 7142 (10 mg/kg) was shown to be behaviorally "equivalent" to 60-90 minutes of uncontrolled tail shock in producing learned helplessness behavior in rats within 24 hours after exposure to the drug. Furthermore, the effects of FG 7142 could be prevented by the administration of the benzodiazepine antagonist Ro 15-1788 which suggests the effects of FG 7142 are produced via

benzodiazepine receptors. These studies provide an intriguing animal model linking anxiety with depression.

The observation that barbiturates (e.g. pentobarbital) are capable of enhancing the apparent affinity of benzodiazepine receptor ligands resulted in a study concluding that the anticonflict actions of pentobarbital may be indirectly mediated through benzodiazepine receptors. Both in vivo and in vitro data suggest that other pharmacologic and toxicologic actions of barbiturates might also be mediated through the benzodiazepine-GABA receptor chloride ionophore complex. Barbiturates such as pentobarbital have been shown to bind to a site on or near the chloride ionophore [demonstrated using both [³H]dihydropicrotoxin and [³⁵S]-butylbicyclophosphorothionate (TBPS)]. Thus, a search was initiated for compounds which could reverse the toxic actions of pentobarbital using the abilities of these substances to act at either the benzodiazepine receptor or chloride ionophore as "leads". It was found that isopropylbicyclophosphate (IPPO), a "cage convulsant" which binds at or near the chloride ionophore, greatly reduced the overall mortality of animals pretreated with a lethal dose of pentobarbital. Picrotoxin, which binds to the same site (and was formerly used as an antidote for barbiturate poisoning) also reduced pentobarbital lethality, but only at doses which were lethal when given alone. Thus, IPPO represents a prototype for development of a "molecular" antagonist of barbiturate action. This observation suggests that compounds which have high affinities for the chloride ionophore in vitro could lead to the development of a specific antidote for clinical treatment of barbiturate toxicity.

Previous studies from this laboratory have shown that the target size of the benzodiazepine receptor (using [³H] diazepam as a radioligand) is approximately 54,000 daltons, while the target size of "peripheral-type" binding sites for benzodiazepine in kidney is approximately 30,000 daltons. Further studies using benzodiazepine antagonist (Ro 15-1788) and an "active" antagonist (β -CCE) as radioligands demonstrated a target size of $51,000 \pm 2,000$. This observation has pharmacologic actions of the ligand used, and thus supports the "domain" "state-transition" theory. Further, the target size for the GABA receptor linked to benzodiazepine receptors is identical to the benzodiazepine receptor. The target size of the chloride ionophore (estimated with [³⁵S] TBPS as a radioligand) was about three fold higher (138,000 daltons). These observations suggest that the GABA and benzodiazepine receptors are physically as well as functionally coupled, and may be physically distinct from the chloride ionophore.

Binding sites for benzodiazepines such as diazepam have also been described in peripheral tissues (e.g. kidney) and transformed cells of neural origin. These "peripheral-type" binding sites (PBS) for benzodiazepines are pharmacologically as well as physically distinct from the "brain-type" benzodiazepine receptors. We previously demonstrated that guinea pig brain is rich in PBS and that Ro 5-4864 (4'-chlorodiazepam), a ligand for this site ($K_d \approx 1$ nM), is a potent convulsant in and rats as well as guinea pigs. Further, Ro 5-4864 increased cell firing in neurons of the substantia nigra zona reticulata at doses well below those needed to elicit convulsions. The pharmacologic profile of agents which are capable of antagonizing both the electrophysiologic and convulsant effects of Ro 5-4864 suggest that "brain-type" benzodiazepine receptors are not directly involved in these actions. However, these studies did not rule out the

possibility that Ro 5-4864 (4'-chlorodiazepam) could be acting at another component of the "supramolecular complex".

Despite the ubiquitous distribution of PBS, the physiologic role in peripheral tissues is still unknown. In the kidney, PBS appear to be localized in the thick ascending limb of Henle (TAL) and the distal convoluted tubule, where the osmolarity may be as high as 1200 mOsm. Thus, studies were initiated to determine whether PBS are involved in ion transport phenomena. Using several sodium and potassium salts, it was found that the apparent affinity of [^3H] Ro 5-4864 was reduced by some anions in crude homogenates of kidney. Iodide was the most powerful anion at reducing the binding of [^3H] Ro 5-4864 while sulfate ions were ineffective. Furthermore, 9-anthroic acid, DIDS and SITS (which affect chloride transport) also inhibited [^3H] Ro 5-4864 binding to kidney homogenates by 30 - 40%, while ouabain was ineffective at concentrations of up to 1 mM. Binding of PK 11195, a quinoline derivative, which has a high affinity for PBS, was little affected by equivalent treatment with either 500 mM iodide or 1 mM DIDS. However, brief treatment of the crude membrane preparation with several detergents rendered PK 11195 susceptible to the influence of both iodide and DIDS. Thus, PBS may play a role in anion transport in the kidney. More recent work has demonstrated a very potent action of benzodiazepines on human macrophage chemotaxis and this effect appears to be mediated by peripheral benzodiazepine receptors. Ro 5-4864 and diazepam are both capable of producing chemotaxis of macrophages and this effect is blocked by PK 11195 the putative PBS antagonist.

Acidified methanol or trichloroacetic acid extraction of membranes from peripheral tissues or brain followed by ultrafiltration and/or gel filtration and high performance liquid chromatography revealed the presence of both high ($M_r > 10,000$ daltons) and low ($M_r > 500$ daltons) molecular weight substances which competitively inhibit the binding of [^3H] Ro 5-4864 to PBS. The binding of [^3H] diazepam to "brain-type" benzodiazepine receptors was only marginally reduced at concentrations of inhibitor which completely inhibited [^3H] Ro-5-4864 to PBS. Within the limited number of tissues surveyed, there appears to be an inverse relationship between the amounts of these endogenous inhibitors and the density of PBS in the same tissue. Separation of the high molecular weight fraction through reverse phase high performance liquid chromatography revealed three discrete regions of inhibitory activity, suggesting the high molecular weight fraction (obtained from a Sephadex G-50 column) consists of at least three components. The chemical nature and physiologic function of these substances remains to be elucidated.

γ -Aminobutyric acid (GABA), the principal inhibitory neurotransmitter in brain, is known to exert its actions by increasing membrane permeability to chloride ions (Cl^-). Many sedative/hypnotic drugs such as the barbiturates also enhance GABA mediated Cl^- permeability as well as directly activating Cl^- ion flux alone. These phenomena have been extensively studied using electrophysiologic techniques but because of methodologic limitations, biochemical studies of Cl^- transport in brain have generally been unsuccessful. Recently, we have examined the effects of various barbiturates and picrotoxin in modifying the efflux of chloride ($^{36}\text{Cl}^-$) in a novel subcellular preparation from rat cerebral cortex, the "synaptoneurosome". Dilution of synaptoneurosome pre-loaded with $^{36}\text{Cl}^-$ resulted in rapid efflux of $^{36}\text{Cl}^-$ that could be measured as

early as 10 sec. following dilution. In the presence of barbiturates such as pentobarbital and hexobarbital there was a significant increase in $^{36}\text{Cl}^-$ efflux which was not observed with the pharmacologically-inactive barbiturate, barbital. The effect of barbiturates in enhancing $^{36}\text{Cl}^-$ efflux was also stereospecific [(-) DMBB > (+) DMBB] and reversed by picrotoxin. Furthermore, the barbiturate-enhanced efflux of contrast, picrotoxin alone significantly inhibited $^{36}\text{Cl}^-$ efflux. These data demonstrate pharmacologically relevant Cl^- transport for the first time in a subcellular brain preparation.

Significance to Biomedical Research and the Program of the Institute:

Many of the psychotherapeutic agents described in these studies are widely prescribed and/or abused substances. Understanding the mechanisms by which these compounds exert their pharmacologic actions are of fundamental importance to a better understanding of anxiety-neuroses, epilepsy, sleep disorders, and depression. These studies may also help clarify the role of ion transport in the mechanisms of action of these agents, and may be of fundamental significance in studies can provide valuable information leading to the development of more efficacious therapeutic agents which lack major side effects.

Proposed Course of Project:

Further behavioral studies are planned with other "atypical" anxiolytics in primates to better define the minimum conditions necessary for an anxiolytic action. The ability to simultaneously monitor endocrine, somatic, and behavioral components of an "anxiety" response in primates following a challenge with β -CCE should prove invaluable in this regard. Studies are also planned to determine the neurochemical correlates of induced learned "helplessness" with an anxiogenic agent. Further studies are planned to assign a physiologic and pharmacologic role to "peripheral-type" binding sites for benzodiazepines both in the brain and peripheral tissues. The pineal gland, previously shown to have a high density of peripheral sites, should be an important model for determining the function of these sites. Studies are planned to further explore the relationship of Ro 5-4964 synaptoneuroosomes as a model system to measure $^{36}\text{Cl}^-$ flux may prove an important tool for this study. Further, structure-activity studies with a number of Ro 5-4864 analogs are in progress to determine the minimum structural requirements necessary for a compound to discriminate "brain" from "peripheral" type benzodiazepine binding sites. A more extensive purification and characterization of endogenous inhibitors of [^3H] Ro 5-4864 binding is also planned.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02186-02 NS

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Brain Recognition Sites
for Stimulants and Antidepressants: Relationship to Pharmacological Activity

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	S. M. Paul	Chief	NS	NIMH
Others:	I. Angel	Guest Researcher	NS	NIMH
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	J. J. Pisano	Staff Fellow	LC	NHLBI
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COOPERATING UNITS (if any)

Lab. of Bioorganic Chemistry, NIAADDK; Section on Molecular Pharmacology, NS, NIMH;
 Sec. on Clinical Studies, NS, NIMH; Clinical Neuropharmacology Branch, NIMH,
 NICHHD, Univ. of Wisconsin; Lab. of Chemistry, Nat. Heart, Lung, and Blood Inst.

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Section on Preclinical Studies

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

5

PROFESSIONAL:

4

OTHER:

1

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Recognition sites for a variety of psychotherapeutic drugs have been identified in the mammalian central nervous system. Several of these binding sites, including those for benzodiazepines, opiates, and various neuroleptics have subsequently been shown to be true pharmacological receptors in that the binding of drug to its respective recognition site is a necessary (and many times sufficient) requirement for drug action. Over the past several years we have attempted to identify recognition sites for other common psychotropic drugs including tricyclic antidepressants and the psychomotor stimulants, amphetamine and methylphenidate.

In each case saturable, and stereospecific binding sites have been delineated; and for amphetamine and methylphenidate relatively good correlations have been observed between the affinities of a series of analogues in vitro and at least some of the pharmacological properties of these agents. Tricyclic antidepressants including imipramine and desipramine, also bind to distinct recognition sites that are functionally and structurally associated with the presynaptic uptake sites for serotonin and norepinephrine respectively. Thus, radiolabelled antidepressants have been useful probes in studying the mechanisms of neurotransmitter uptake in both central and peripheral tissues, and under a variety of clinical conditions. More recent work suggests that the [³H] (+)-amphetamine binding site in hypothalamic membranes is sensitive to circulating levels of blood glucose. Hypoglycemia decreases, and hyperglycemia increases, the number of [³H] (+)-amphetamine binding sites in hypothalamic membranes respectively. Furthermore, these changes seemed to be coupled to the activity of (Na⁺ K⁺) ATPase; and there is a good correlation between the changes in [³H] (+)-amphetamine and [³H]-ouabain binding both in vivo and in vitro.

Other Professional Personnel:

J. N. Crawley	Res. Biologist	NS NIMH
R. D. Schwartz	Pharmacologist	NS NIMH
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M. L. Caspers	Guest Researcher	NS NIMH

Project Description:Methods Employed:

Radioreceptor techniques using radioactive ligands of high specific radioactivity have been employed for the neurochemical identification and characterization of specific drug recognition sites. In vitro experiments employing brain tissue slices incubated under optimal incubation conditions are used to study the metabolic influences on [³H] (+)-amphetamine binding. Behavioral studies in rodents are conducted to compare the pharmacological (viz. behavioral) potencies of drugs with their in vitro potencies at their respective binding sites. In vitro neurotransmitter uptake studies conducted in crude and partially purified synaptosomal and platelet fractions are performed as a functional measure in studies where drugs that potently block reuptake (e.g. antidepressants, methylphenidate and amphetamines) are being studied.

Major Findings:

High affinity, stereospecific binding sites for [³H] (+)-amphetamine have been previously described in rodent brain. The highest density of these sites are found in the synaptosomal fraction of brain stem and hypothalamus. A striking correlation ($r = 0.97$; $p < .01$) has been demonstrated between the ability of a series of amphetamine derivatives in displacing [³H] (+)-amphetamine from these sites and their potencies as anorectic agents. A similar correlation was not observed between their in vitro potencies and their behavioral potencies as motor stimulants. These observations suggested that this site may be involved in the appetite suppressant actions of amphetamine. [³H] (+)-Amphetamine binding has also been studied in a genetically obese mouse strain, Ob/Ob. In these animals, the density of hypothalamic [³H] (+)-amphetamine binding sites is greater than in lean litter mate controls. Furthermore, food deprivation of rats (24-72) hours results in a dramatic (30-50%) reduction in the density of hypothalamic [³H] (+)-amphetamine binding sites. Refeeding food-deprived animals for a four hour period (or allowing access to a 10% glucose solution) results in a return of [³H] (+)-amphetamine binding site density to control values. These data suggest that the [³H] (+)-amphetamine binding sites may be intimately involved in the regulation of feeding behavior of animals.

Since [³H] (+)-amphetamine binding in hypothalamus is decreased following 24 hours of food deprivation (30% reduction in B_{max}), and the site density restored to control levels if the animals are permitted to refeed for four hours we have examined the factors responsible for this rapid modulation in site number. More recent studies have now demonstrated that intraperitoneal injection of D-glucose elicits a significant increase in [³H] (+)-amphetamine binding in the hypothalamus. This treatment did not alter [³H] (+)-amphetamine binding in other brain regions.

Injection of L-glucose failed to elicit an increase in [^3H] (+)-amphetamine binding. Further, injection of 2-deoxyglucose coupled with food deprivation elicited a similar increase in binding site density. However, if animals are permitted access to food during the four hour interval following injection of the 2-deoxyglucose, the increase in [^3H] (+)-amphetamine binding is not observed. These observations suggest that [^3H] (+)-amphetamine binding sites in the hypothalamus are coupled to glucose utilization and/or transport. The high correlation previously reported between the ability of a number of phenethylamines to inhibit [^3H] (+)-amphetamine binding and their potencies as anorectics may thus link the anorectic actions of phenethylamines with their ability to effect glucose-response neurons in the hypothalamus. In related experiments the regulation of [^3H] (+)-amphetamine binding in hypothalamic tissue slices *in vitro* have confirmed that glucose plays a major role in determining the density of [^3H] (+)-amphetamine binding sites. In the absence of glucose, incubation of hypothalamic slices in physiological buffer at 37°C results in a time-dependent decrease in [^3H] (+)-amphetamine binding whereas the addition of glucose (1 - 30 mM) significantly stimulates binding in crude synaptosomes prepared from the incubated slices. These changes are again highly correlated to similar changes in high affinity [^3H]-ouabain binding; indicating a relationship to the neuronal form of ($\text{Na}^+ \text{K}^+$) ATPase.

Previous studies from this laboratory have demonstrated the presence of high affinity, stereospecific binding sites for [^3H] (\pm) threo-methylphenidate in the striatum and brainstem of the rat. Subsequent studies have demonstrated that the binding of [^3H] methylphenidate is localized to synaptosomes, and that the binding is dependent on the presence of sodium. Intraventricular administration of 6-hydroxydopamine or medial forebrain bundle lesions results in a significant loss of [^3H] methylphenidate binding in striatum which is highly correlated with a loss in the capacity of this tissue to take up [^3H] dopamine. Structure-activity studies suggest that this site is associated with a dopamine transport system since a high correlation ($r = 0.88$, $p < .001$) was found between the potencies of a series of compounds to inhibit [^3H] dopamine uptake and inhibit [^3H] methylphenidate binding. These findings suggest the methylphenidate binding site may be part of a dopamine "transporter", analogous to findings demonstrating that [^3H] imipramine and [^3H] desipramine label components of the serotonin and dopamine "transporters", respectively. Further, pilot studies in human autopsy material suggest a distribution of site density similar to that found in rat, the highest site densities in striatum, and lowest in cerebellum. In a related series of experiments several diphenyl-substituted piperazines (GBR-12935, GBR-12921) have been tested for their selectivity in inhibiting dopamine uptake. The marked specificity of these compounds in inhibiting dopamine uptake has prompted the radioactive labelling of GBR-12935. [^3H] GBR-12935 appears to be a "super high affinity" ligand for the dopamine uptake site and may be useful for *in vivo* imaging of dopamine-containing neurons. Previous work in our laboratory has demonstrated that [^3H] imipramine labels the "serotonin transporter" (recognition site + transport protein) in brain and platelets of both human and rat. Since the number of these sites is reduced in platelets of depressed patients, this parameter could be an important marker for depressive illness. The demonstration of a strong concordance of [^3H] imipramine binding to platelets from monozygotic (identical) twins further supports the contention that this parameter may be a useful "biological marker"

in depression. In followup of our initial work on [^3H] imipramine binding we have successfully solubilized the [^3H] imipramine binding site from human platelets with the ultimate goal of reconstitution of the serotonin transport system in artificial membranes. We have also immunized BALB/C mice with a partially purified human platelet membrane preparation and have generated monoclonal antibodies directed against platelet membranes. A number of these antibodies cross-react with rat brain synaptosomal membranes and alter serotonin uptake and [^3H] imipramine binding in this tissue as well. Studies are in progress to characterize the most interesting monoclonal antibodies and to identify whether they are specifically directed against the serotonin transporter and (or) [^3H] imipramine binding site. The presence of specific recognition sites for [^3H] imipramine and [^3H] desipramine that are associated with the presynaptic transport mechanisms for serotonin and norepinephrine respectively, have prompted a series of studies on the possible presence of an endocoid(s) that may regulate uptake via these sites. Since preliminary studies revealed that plasma extracts potently inhibited the uptake of serotonin in crude synaptosomal membranes, studies on the purification of such a factor(s) from plasma were initiated. To date, at least two active inhibitory fractions have been characterized from human and rat plasma using reverse phase HPLC. These fractions are selective in that they both inhibit [^3H] imipramine binding and serotonin uptake more potently than they inhibit the uptake of a variety of other neurotransmitters (e.g. norepinephrine, GABA). Pharmacological experiments have also demonstrated that the quantity of at least one fraction can be altered by administration of drugs known to alter the synaptic concentration of serotonin. Future studies will focus on the further purification of these factor(s).

The hydrolysis of phosphatidylinositol (PI) or the polyphosphoinositides is now recognized as a "second messenger" system mediating signal transduction for a variety of hormones and neurotransmitters. PI "turnover" is dramatically altered by lithium, since the latter is a rather potent inhibitor of inositol phosphatase and thus the recycling of inositol. We, and others, have capitalized on the use of lithium to amplify the effects of various neurotransmitter agonists in causing an accumulation of inositol-1-phosphate in vitro. Thus, using brain slice preparations we have characterized the effects of various neurotransmitters in stimulating PI hydrolysis and have used this system for studying the regulation of PI-coupled receptors. To date, we have examined the effects of various chemical and surgical lesions on neurotransmitter-mediated PI turnover in discrete brain regions. In addition, we have observed that activation of protein kinase C by phorbol esters markedly inhibit the neurotransmitter-stimulated turnover of PI; indicating the possible existence of a negative feedback loop. The characterization of a suitable in vitro system for studying PI turnover should prove useful in studying the regulation of the various receptors coupled to PI turnover.

Proposed Course of the Project:

Studies will continue on the various recognition sites described above to more fully elucidate their pharmacological as well as physiological significance. A major emphasis will be placed on defining the alterations in [^3H] (+)-amphetamine binding that occur in vivo during various manipulations of "appetite" and "satiety", in order to test the hypothesis that these sites are

coupled to a physiological mechanism regulating food intake (particularly carbohydrate intake) in animals. The relationship between the [^3H] (+)-amphetamine binding and the neuronal form of $\text{Na}^+ \text{K}^+$ ATPase will also be investigated since the latter is one of the most important "utilizers" of energy derived from glucose. A variety of other chromatographic methods will be used to characterize and purify the [^3H] imipramine/[^3H] desipramine recognition sites. The production of monoclonal antibodies against many of these proteins are in progress and their use should be valuable in their further characterization and purification.

Significance to Biomedical Research and the Program of the Institute:

All of the drugs under investigation have important psychotropic actions and are either of therapeutic benefit or reliably mimic various behavioral states. Thus an understanding of their mechanisms of action will be of undoubted value to understanding the behavioral and psychopathological states responsive to treatment with these agents.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00112-07 NS

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Endorphin Research in Mental Illness

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: D. Pickar Chief, Section on Clinical Studies NS, NIMH

Others: G.A. Roy Visiting Associate NS, NIMH
 O.M. Wolkowitz Medical Staff Fellow NS, NIMH
 M.R. Cohen Staff Psychiatrist NS, NIMH
 R.M. Cohen Chief, Clinical Brain Imaging Section LPP, NIMH
 P. Sugarbaker Head, Colorectal Cancer Section SB, NCI
 T.N. Wise Chief of Psychiatry, Fairfax Hospital

COOPERATING UNITS (if any)

Laboratory of Psychology and Psychopathology, NIMH; Surgery Branch, National Cancer Institute; Georgetown Medical School; Fairfax Hospital

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Section on Clinical Studies

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

4.5

PROFESSIONAL:

2.5

OTHER:

2.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project studies the role of the endogenous opioid system (EOS) in humans. We have completed a dose-response study in normals of high doses of the opiate antagonist, naloxone (up to 4 mg/kg). Significant dose-dependent increases in physiologic (BP and respiratory rate) and hormonal variables (cortisol and growth hormone) were found suggesting progressive EOS blockade with increasing naloxone doses. Normals also experienced dysphoria at high naloxone doses suggesting EOS involvement in the regulation of mood in normals. In a separate double-blind study, high doses of naloxone produced a significant decrease in caloric intake in healthy, normal volunteers supporting hypothesized involvement of the EOS in eating behavior. In other work, we have studied the relationship between the hypothalamic-pituitary-adrenal axis and the EOS in depressive illness. As a continuation of our work demonstrating the responsiveness of the EOS to stress and involvement of the EOS in endogenous analgesic mechanisms, we have initiated a study of the analgesic and behavioral effects of synthetic β -endorphin administered by lumbar intrathecal route to patients with metastatic disease. In the two patients studied to date, intrathecally administered β -endorphin produced long-lasting analgesia. In addition, one of the patients experienced a unique behavioral syndrome characterized by hypomania/mania, psychosis and confusion. Further work is needed with this strategy to more fully evaluate analgesic potentials and the behavioral effects of this endogenous peptide when administered with access to the CNS.

OTHER PROFESSIONAL PERSONNEL

M. Dubois Staff Member, Department of Anesthesia, Georgetown Medical School

PROJECT DESCRIPTION

The major aim of this project is to study the roles of the endogenous opioid system (EOS) in human behavior and physiology and in psychiatric illness. We have used naloxone administration strategy to study the tonic role of the EOS in humans. We have further studied links between hypothalamic-pituitary-adrenal (HPA) axis and the EOS in depressed patients. Finally, we have continued to investigate the response of endogenous opioids to stress and its relationship with endogenous analgesic mechanisms.

METHODOLOGY

A. The Administration of High-Dose Naloxone

We have developed the methodology of administering larger doses of the opiate antagonist, naloxone, than had been previously administered in the clinical setting. Doses up to 4 mg/kg have been administered in a dose-response study to normal volunteers. Dose-response changes in behavior, physiologic function and neuroendocrine variables have been examined to gain a better understanding of the optimal dose of naloxone to ensure EOS blockade and the tonic role of endorphins in humans. We have used a standard 0.5 mg/kg dose of naloxone to study the response of the HPA axis to endogenous opioid system blockade in psychiatric illness.

B. Plasma Measurements

We have continued to use the strategy of measuring plasma levels of β -endorphin immunoreactivity (ir) in the clinical setting, particularly to study relationships between levels of plasma β -endorphin (ir) and levels of plasma cortisol in depression.

C. Intrathecal β -Endorphin Administration

We have previously demonstrated that the EOS, as reflected by levels of plasma β -endorphin (ir), is extremely responsive to the severe physical stress of abdominal surgery. We, furthermore, demonstrated that levels of β -endorphin (ir) stimulated by surgical stress predicted (inversely) the amount of post-operative analgesic required to control the patient's pain. We have continued our interest in endogenous analgesic and stress aspects of the EOS. Towards this end, we have developed, in collaboration with the National Cancer Institute, a protocol in which 3 mg of synthetic β -endorphin is administered intrathecally (lumbar route) to patients with severe pain secondary to malignancy. This project intends to study the reported high-potency analgesic effects of β -endorphin when so administered as well as the behavioral effects of this endogenous peptide when administered in pharmacologic doses with access to the CNS. This project requires intensive medical monitoring and is carried out in a surgical intensive care unit of the NIH Clinical Center.

MAJOR FINDINGS

A. Previous clinical studies using the opiate receptor antagonist, naloxone, have shown little or inconsistent behavioral effects in normal humans. In order to assess the notion that previous doses used were insufficient to yield a complete EOS blockade, normal volunteers were administered increasing doses of naloxone (0.3-4.0 mg/kg) in a single-blind study. We have observed significant dose-dependent behavioral, hormonal (cortisol and growth hormone) and physiological effects associated with increasing doses of naloxone. With high naloxone doses, volunteers experienced increasing dysphoria, a deterioration of performance on memory testing, increased systolic blood pressure and respiratory rate. These results suggest that lower doses of naloxone used in previous clinical studies may not have been sufficient to produce complete EOS blockade, and indicate involvement of EOS in the tonic regulation of normal human mood, memory, BP, respirations, plasma growth hormone and cortisol levels. In a separate double-blind study using a 2 mg/kg dose of naloxone administered to 7 normal volunteers, naloxone significantly reduced total food intake from preselected prepared trays served at 2.75 and 7.75 hours following drug administration. These data are consistent with animal studies demonstrating EOS modulation of food intake and suggest further studies for use of naloxone in treating eating disorders. Data concerning the effects of naloxone in obese subjects is not yet complete.

B. We have measured plasma β -endorphin in groups of patients with major depressive and minor depressive disorder and normal controls. Minor depressives had significantly less plasma β -endorphin than did patients with major depression or controls. In contrast, patients with major depressive disorder demonstrated predictable elevations in plasma cortisol compared to the other study groups and in major depressives, but not other groups, plasma β -endorphin was directly correlated with plasma cortisol. This finding complements earlier work from our group suggesting a link between the HPA axis and the EOS in major depression.

We have also studied the relationship between the EOS and HPA axis in obesity in collaboration with Fairfax Hospital. Morning plasma cortisol and β -endorphin levels were found to be no different in obese patients, prior to diet treatment, than normal weight relatives. Plasma cortisol levels were significantly correlated in obese patients with self-ratings of depression. During the course of a 400 calorie per day modified protein fast we observed significant decreases in levels of plasma cortisol but unchanging levels of plasma β -endorphin. Patients who failed to complete the 6-month diet program, however, were found to have had significantly higher levels of plasma β -endorphin on entry into the program than those who were able to complete the diet program.

C. We have administered 3 mg of β -endorphin, by lumbar intrathecal route, to 2 patients with disseminated malignancy. We observed that β -endorphin produced profound and long-lasting analgesia. The first patient experienced analgesia lasting over 16 hours; the second patient experienced analgesia lasting over 60 hours. Though there was a suggestion of enhanced mood in the first subject, the second subject experienced a significant behavioral

effect. This behavioral syndrome was characterized by confusion, hypomanic/ manic behavior and psychosis and lasted for over 2 days. We are continuing to evaluate this methodology for future studies in which both the analgesic and behavioral effects of β -endorphin are to be studied.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND TO THE PROGRAM OF THE INSTITUTE

The endogenous opioid system represents one of the most important scientific discoveries of the last decade. Our program, aiming to discern the roles of this system in humans, is of considerable importance not only to psychiatry but to the overall field of medicine. Our work, using high doses of naloxone has added to methodologic approaches in studying the endogenous opioid system and has provided new evidence suggesting tonic roles for this system in human behavior, physiology and neuroendocrine regulation. We, furthermore, have evidence suggesting a role for the endogenous opioid system in eating behavior in normal subjects.

Our work in psychiatric patients has continued to suggest important relationships between the HPA axis and the EOS in depression. This work is of particular significance since abnormalities in HPA axis function have been a consistent and to date unexplained finding in depression.

The strategy of intrathecal β -endorphin administration is important for several reasons. First, it represents an aggressive treatment/research technique in which substances (e.g., peptides) which do not readily cross the blood-brain barrier following peripheral administration can now be studied with regard to CNS effects. Furthermore, it appears that β -endorphin itself may be an extremely potent analgesic which may alleviate suffering in patients with chronic and severe pain. Furthermore, our data support the notion that β -endorphin itself may have important behavioral properties in humans.

PROPOSED COURSE

We intend to continue our studies using naloxone both in normal and abnormal states. Furthermore, we intend to utilize naloxone strategies in depressed, schizophrenic and normal subjects with particular attention to differential HPA axis responses between groups. Finally, the difficult but potentially important strategy of intrathecal drug administration will be pursued with the National Cancer Institute.

PUBLICATIONS

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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02181-02 NS

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neurobiology of Schizophrenia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: D. Pickar Chief, Section on Clinical Studies NS, NIMH

Others: S.M. Paul Chief NS, NIMH
 G.A. Roy Visiting Associate NS, NIMH
 O.M. Wolkowitz Medical Staff Fellow NS, NIMH
 A.R. Doran Medical Staff Fellow NS, NIMH
 J.L. Schreiber Social Worker NS, NIMH
 M. Linnoila Clinical Director ALC, NIAAA

COOPERATING UNITS (if any)

Alcohol Intramural Research Program, National Institute of Alcohol Abuse and Alcoholism; Laboratory of Psychology and Psychopathology, NIMH; Adult Psychiatry Branch, St. Elizabeths Hospital, NIMH

LAB/BRANCH

Clinical Neuroscience Branch

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Section on Clinical Studies

INSTITUTE AND LOCATION

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TOTAL MAN-YEARS:

3.5

PROFESSIONAL:

2.5

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The aim of this project is to gain a greater understanding of the psychobiology of schizophrenia. Clinical research for this project is carried out on the 4-East Nursing Unit of the Clinical Center. Patients with DSM-III diagnosed schizophrenia are studied during a several month research period during which time they are treated under double-blind conditions with neuroleptic and placebo medications. A major goal of this project is to gain a better understanding of the mechanism of action of neuroleptic drugs and a profile of which symptoms are most responsive to drug treatment. Data from a preliminary study have shown that neuroleptic-induced, time-dependent decreases in levels of plasma HVA (a major dopamine metabolite) are correlated with antipsychotic drug response. These data suggest that neuroleptic effects on dopamine release are related to clinical improvement. These findings are being more fully examined using hourly blood sampling during 24-hour studies, and may be a useful model for studying pharmacologic profiles of potential antipsychotic drugs. Preliminary investigations into the possible therapeutic effect of the calcium channel blocker, verapamil, are underway since dopamine release and postsynaptic transmission are calcium dependent phenomena.

OTHER PROFESSIONAL PERSONNEL

A. Mirsky	Chief	LPP, NIMH
R.M. Cohen	Chief, Clinical Brain Imaging Section	LPP, NIMH
R. Coppola	Senior Engineer	LPP, NIMH
D. Weinberger	Chief, Section on Clinical Neuropsychiatry	SMRA, NIMH
R. Zec	Staff Fellow, Section on Clinical Neuropsychiatry	SMRA, NIMH

PROJECT DESCRIPTION

This project is part of the research program of the Section on Clinical Studies of the Clinical Neuroscience Branch. This section conducts clinical research based on the 4-East Nursing Unit of the Clinical Center.

Despite enormous research and clinical efforts to alleviate symptoms of schizophrenia, the group of drugs known as neuroleptics introduced over the last two decades have remained as the principle pharmacologic agents for the treatment of schizophrenia. The close relationship between the affinities of representative neuroleptic drugs to bind to non-adenylcyclase dependent postsynaptic dopamine receptors and their clinical antipsychotic potencies represents the foundation of the dopamine hypothesis of schizophrenia. This research program has attempted to study the mechanism of neuroleptic action in man with particular attention to changes in dopamine system activity and clinical response. The goal is to improve our understanding of the pathophysiology of schizophrenia and to provide the foundation for developing improved forms of treatment.

METHODOLOGY

A. Behavioral Assessment

Patients are rated daily by the 4-East nursing staff using the Bunney-Hamburg Scale. Patients are maintained on double-blind medication throughout the research period which includes at least 4 weeks free from all medications. In addition to nurses' ratings, physicians, blind to patients' medication status, perform Bunney-Hamburg global ratings of psychosis and depression, the Brief Psychiatric Rating Scale, and the Taylor-Abrams Scale for the assessment of negative symptoms.

B. Plasma and CSF Monoamine Metabolites

During the course of neuroleptic and placebo administrations, plasma is collected three times weekly for assessment of levels of monoamine metabolites. HVA, a major metabolite of dopamine, is examined with regard to the course of behavioral change associated with neuroleptic treatment. Levels of plasma MHPG and 5HIAA are also examined during selected periods with particular emphasis in studying their relationship to affective symptoms.

Based on our preliminary results, we have initiated a series of 24-hour studies during which we sample blood hourly to assess levels of amine metabolites. This methodology will enhance our ability to examine neuroleptic effects on levels of neurotransmitters as well as the effects of circadian rhythms and of the sleep-wake cycle.

In addition to these plasma measurements, at least two lumbar punctures (while the patient is on and off neuroleptics) are performed during the patient's hospitalization. CSF amine metabolites, norepinephrine and dopamine levels are determined and examined for possible relationships to behavioral change.

C. New Drug Treatments of Schizophrenia

One important aspect of this study is the development of improved treatment strategies for patients with schizophrenia. We have examined the effects of ceruletide, a synthetic analogue of cholecystokinin, a naturally occurring peptide known to interact with dopamine cells in the CNS. This study was performed with double-blind methodology in medication-free and neuroleptic-treated schizophrenic patients.

We have recently begun a double-blind crossover study of the effects of the calcium channel blocker, verapamil, on the symptoms of schizophrenia. This drug impedes the entry of calcium via certain calcium channels and may thus have effects on neurotransmitter release or calcium-dependent "second messenger" function. Biochemical and clinical effects of verapamil will be compared with those of the neuroleptic, fluphenazine. We also are utilizing brain imaging techniques such as regional cerebral blood flow and brain electrical activity mapping to study CNS effects of verapamil.

D. CAT Scan Studies

Studies from centers throughout the world have now reported the occurrence of abnormalities in ventricular size in patients with schizophrenia. We have utilized CAT scan technology to study the size of lateral and third ventricles using planimetric methods and have determined sulcal atrophy in a sizable group of patients with schizophrenia and in a matched group of medical controls from the Clinical Center. CAT scan findings will be examined for possible relationship to neuroleptic response.

E. PET Scan

Positron emission tomography, one of the newest technologies applicable to studying brain function, is utilized as part of our research program. In collaboration with the Laboratory of Psychology and Psychopathology, we are developing better methods for studying patients with schizophrenia during PET scan procedures. Comparison of glucose utilization between medication-free and neuroleptic-treated schizophrenics is in progress.

F. Neuropsychological Testing

In collaboration with the Adult Psychiatry Branch at St. Elizabeths Hospital we are administering a battery of neuropsychological tests to patients with schizophrenia. This work is intended to document and describe significant neuropsychological dysfunction in patients with schizophrenia. These data are to be examined with regard to drug response, biochemical measures and performance on neurophysiologic tests.

G. "Positive" and "Negative" Symptomatology in Schizophrenia

It has been appreciated for many years that patients with schizophrenia suffer both from "positive" symptoms (hallucinations, delusions, etc.) as well as "negative" symptoms (social withdrawal, poverty of speech, etc.). This portion of our research program studies "positive" and "negative" symptoms in schizophrenia and their relationship to drug response and biochemical measures. Data from behavioral rating scales are used for this purpose (see Item A).

MAJOR FINDINGS

An important finding has emerged from our longitudinal studies of plasma amine metabolites. We have observed a time-dependent decrease in the levels of plasma HVA during neuroleptic treatment. Further, the decrease in plasma HVA, occurring after 3 weeks of treatment, correlates with the antipsychotic response to neuroleptic treatment. These data suggest that changes in the release of dopamine may be more closely related to neuroleptic response than blockade of dopamine transmission. This pattern of neuroleptic effects on plasma HVA may provide a marker for neuroleptic response and a better insight into pharmacologic mechanisms of action of this group of drugs.

Preliminary results from studies using multiple blood sampling over a 24-hour period substantiates the notion that chronic neuroleptic treatment decreases levels of plasma HVA. When completed, these data may also provide an opportunity of examining variability in release of dopamine in schizophrenic patients compared with normal subjects.

A recently completed CAT scan study has demonstrated larger III ventricles in schizophrenic patients than in matched medical controls. We were unable to demonstrate, however, a relationship between ventricular size (lateral or IIIrd) and either neuroleptic response or biochemical measures.

We were unable to demonstrate any behavioral effects of parenterally-administered CCK or ceruletide in schizophrenic patients.

At the present time the pilot study of verapamil treatment of schizophrenic patients is underway. Initial experience suggests that this drug is well tolerated by patients and may have some therapeutic effect. Completion of this study, including the development of a thorough pharmacologic profile of verapamil effects on levels of neurotransmitters in CSF and plasma is anticipated during the forthcoming year.

Systematic study of "positive" and "negative" symptoms of schizophrenia are moving towards completion. Preliminary results suggest that both types of symptoms occur concurrently in many patients with schizophrenia and respond in parallel to treatment with antipsychotic drugs. Relationships between these symptoms and levels of neurotransmitter metabolites in CSF and plasma are currently under examination.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND TO THE PROGRAM OF THE INSTITUTE

Schizophrenia is a major public health problem in the United States. This project attempts to study possible etiologic factors in schizophrenia and to develop a better understanding of mechanisms underlying current pharmacologic treatments. Our finding of time-dependent decreases in plasma HVA during neuroleptic treatment for the first time relates effects of neuroleptics on presynaptic dopamine function to antipsychotic effects. This pharmacologic effect may prove to be a marker for antipsychotic drug response. The pilot study of verapamil is the first controlled study to our knowledge examining the effect of calcium channel blockade in schizophrenia. New treatment strategies which may develop from this work would have considerable importance to the field of psychiatry and to the estimated 2 million patients suffering from schizophrenia in the United States.

PROPOSED COURSE

We will be examining data for promising leads and new directions with the explicit goal of developing better treatments for schizophrenia. Our interest in using plasma HVA as a marker for psychosis and/or antipsychotic drug response has led us to develop pharmacologic challenge strategies to the dopamine system. These include methylphenidate and apomorphine infusions to provide better understanding of dopamine system activity in schizophrenia. Results from the study of calcium channel blockade will be evaluated with regard to future drug treatment strategies.

PUBLICATIONS

Boronow, J., Pickar, D., Ninan, P. T., Roy, A., Hommer, D., Linnoila, M., and Paul, S. M.: Atrophy limited to IIIrd ventricle only in chronic schizophrenic patients: report of a controlled series. Arch. Gen. Psychiatry (in press).

Hommer, D. W., Pickar, D., Roy, A., Ninan, P., Boronow, J., and Paul, S. M.: The effects of ceruletide in schizophrenia. Arch. Gen. Psychiatry 41: 617-619, 1984.

Pickar, D., Labarca, R., Linnoila, M., Roy, A., Hommer, D., Everett, D., and Paul, S. M.: Neuroleptic-induced decrease in plasma homovanillic acid and antipsychotic activity in schizophrenic patients. Science 225: 954-957, 1984.

Roy, A., Mazonson, A., and Pickar, D.: Attempted suicide in chronic schizophrenia. Br. J. Psychiatry 144: 303-306, 1984.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02184-02 NS

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neurobiology of Depression

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

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Others: S.M. Paul Chief NS, NIMH
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 O.M. Wolkowitz Medical Staff Fellow NS, NIMH
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 W.Z. Potter Chief, Unit on Clinical Psychopharmacology CP, NIMH

COOPERATING UNITS (if any) Alcohol Intramural Research Program, National Institute of Alcohol Abuse and Alcoholism; Clinical Psychobiology Branch, Biological Psychiatry Branch and Laboratory of Neurochemistry, NIMH; Hypertension-Endocrine Branch, National Heart, Lung and Blood Institute

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Section on Clinical Studies

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

4.5

PROFESSIONAL:

2.5

OTHER:

2.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The aim of this study is to investigate selected areas of the neurobiology of depression. Depressed patients free of psychotropic medications for at least two weeks are evaluated, diagnosed and symptoms rated on the 4-East Clinical Research Unit of the NIH Clinical Center. During the past year we have attempted to assess neurotransmitter function in depression by measuring levels of amine and amine metabolites in plasma and cerebrospinal fluid in depressed patients and normal controls. We have observed higher levels of circulating plasma norepinephrine in unipolar patients with DSM-III diagnosed melancholia in comparison to controls. We have also found that melancholic patients have lower levels of CSF HVA and DOPAC than nonmelancholic depressed patients. These data suggest activation of the sympathetic nervous system and diminished CNS dopaminergic system function, respectively, in patients with melancholia. We have also studied underlying mechanisms involved in the activation of the hypothalamic-pituitary-adrenal (HPA) axis, a disturbance found in many depressed patients. We have observed that nonsuppression to the dexamethasone suppression test (DST) is associated with higher levels of plasma norepinephrine and with higher levels of CSF MHPG. We have also found that low levels of the brain peptide, somatostatin, are associated with DST nonsuppression. We have also investigated clinical and biochemical effects of tetrahydrobiopterin, a pterin cofactor, in a pilot study of depressed patients.

OTHER PROFESSIONAL PERSONNEL

D. Rubinow	Chief, Unit on Peptide Studies	BPB, NIMH
P.W. Gold	Chief, Unit on Clinical Neuroendocrinology	BPB, NIMH
S. Kaufman	Chief	LNC, NIMH
W. Lovenberg	Chief, Section on Biochemical Pharmacology	HE, NHLBI

PROJECT DESCRIPTION

The purpose of this project is to investigate selected neurobiological aspects of depressive illness. Towards this end we have studied a spectrum of patients with depression in a short-term intensive program on the 4-East Clinical Research Unit of the NIH Clinical Center. After at least 14 days free of all medications, patients are studied using a variety of methodologies including the assessment of levels of monoamine and monoamine metabolites in plasma and cerebrospinal fluid, and investigations of biological mechanisms underlying hyperactivity of the hypothalamic-pituitary-adrenal axis in depression. This is a continuation of the previous project "Biological Tests in Depression" (Z01 MH 02184 01).

METHODOLOGY

A. All patients receive a thorough clinical evaluation including diagnosis by DSM-III criteria. Symptomatology is quantified using a number of well-established rating scales. In addition, the presence or absence of life events which may have occurred during the six months prior to the onset of depression is recorded.

B. Catecholamines and their Metabolites

A thorough evaluation of neurotransmitter function is undertaken in all depressed patients. This evaluation includes the determination of circulating levels of plasma norepinephrine (NE) and of plasma HVA and MHPG. Levels of norepinephrine, dopamine sulphate, HVA, DOPAC are also determined in cerebrospinal fluid (CSF). Urinary levels of MHPG and other NE metabolites are also determined.

C. Assessment of HPA Axis Function

HPA axis activity is assessed by determining levels of urinary free cortisol, plasma cortisol, and the cortisol response to dexamethasone (dexamethasone suppression test). Systematic evaluation of biologic phenomena associated with HPA axis activity is made with particular attention to catecholamines and the peptide, somatostatin. Pharmacologic challenges to aid in evaluating HPA axis activity include the administration of CRF, the opioid antagonist, naloxone, and the serotonin precursor 5-HTP.

D. Drug Trials

We have completed a pilot investigation in five patients of the clinical and biochemical effects of tetrahydrobiopterin (BH₄), a pterin cofactor required in the synthesis of catecholamines. After at least 14 days

medication-free, patients are begun on a double-blind placebo controlled study of oral BH₄ (1 gm/day for 1 week; 2 gm/day for 2 weeks). Plasma and CSF levels of catecholamine are examined with regard to effect of BH₄ treatment. We have also begun to investigate the reported synergistic relationship between the tricyclic antidepressant, imipramine, and lithium.

MAJOR FINDINGS

- A. We have observed elevated levels of plasma norepinephrine in patients with DSM-III diagnosed melancholia in comparison to controls. Investigation into bipolar-unipolar differences revealed that elevated plasma NE levels are restricted to patients with unipolar illness. Preliminary data suggests that levels of plasma HVA may be decreased in patients with depression in comparison to patients with schizophrenia and controls. This finding is similar to our observation that patients with melancholia have lower levels of HVA and DOPAC in CSF in comparison with nonmelancholic patients. We have been unable to replicate previous reports of lower levels of CSF 5-HIAA in patients with depression or those with a history of suicide. Preliminary data does suggest that patients with lowest levels of dopamine metabolites in the CSF are those whose depressive illness was not preceded by the occurrence of life events. Further analysis is underway on the levels of urinary catecholamine metabolites and of their relationship to levels of metabolites in plasma and CSF.
- B. Several lines of evidence from our studies have suggested that enhanced sympathetic nervous system arousal is associated with hyperactivity of the HPA axis. We have observed higher levels of plasma norepinephrine and higher levels of CSF MHPG (but not norepinephrine) in depressed patients who are nonsuppressors to DST. DST nonsuppression was also found to be associated with lower levels of CSF somatostatin, an important brain peptide which is thought to modulate ACTH release. We are currently evaluating the results of challenge paradigms using naloxone, CRF, and 5-HTP administration to depressed patients and evaluating the activity of the HPA axis.
- C. Results from the pilot study of BH₄ administration to depressed patients have revealed no clear therapeutic benefit. Levels of plasma HVA and MHPG appear to increase during BH₄ treatment, although no changes were observed in CSF. Evaluation of BH₄ dosage, route of administration and duration of treatment are currently under consideration in developing future protocols with this compound. Preliminary data has suggested that imipramine treatment increase levels of plasma HVA. In several patients we have observed clinically meaningful antidepressant effects by the addition of lithium. We are currently evaluating strategies to study the mechanism(s) of this effect.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND TO THE PROGRAM OF THE INSTITUTE

In this project we have focused on selected areas of neurobiology pertaining to depressive illness. Our findings of enhanced noradrenergic system activity in depression replicate and extend those reported by other groups; the association between plasma NE and CSF MHPG and DST nonsuppression are new findings and may help explain variability in levels of these measures which have been reported in

groups of depressed patients. A better understanding of the mechanisms underlying HPA axis activity in depression is an important goal for biological research in depression. Our finding with CSF somatostatin and other work involving pharmacologic challenge strategies may extend our knowledge of this important biological phenomena. The evaluation of new or improved treatments may potentially impact on the clinical care of depressed patients.

PROPOSED COURSE

We will continue to study medication-free depressed patients with regard to levels of plasma norepinephrine in comparison to other psychiatric illnesses. We plan to perform circadian rhythm studies in which we determine levels of plasma HVA and MHPG in depressed patients in order to better assess neurotransmitter function and to make comparisons with other psychiatric illnesses such as schizophrenia. Results from pharmacologic challenge strategies will help to provide new directions of research.

PUBLICATIONS

Roy, A., Pickar, D., and Paul, S. M.: Biological tests in depression. Psychosomatics 25: 443-454, 1984.

Roy, A., Sutton, M. S., and Pickar, D.: Dysthymic disorder: neuroendocrine and personality variables. Am. J. Psychiatry (in press).

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02187-01 NS

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Diazepam Infusions as a Measure of Benzodiazepine Receptor Sensitivity in Humans

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

I: D.H. Hommer Staff Psychiatrist NS, NIMH

Others: S.M. Paul Chief NS, NIMH
 D. Pickar Chief, Section on Clinical Studies NS, NIMH
 O.M. Wolkowitz Medical Staff Fellow NS, NIMH
 H. Weingartner Chief, Section on Psychopathology LPP, NIMH
 V. Matsuo Physiologist IR, NEI
 G. Chrousos Medical Staff Fellow IR, NEI

COOPERATING UNITS (if any)

Laboratory of Psychology and Psychopathology, NIMH; Intramural Research, National
 Eye Institute; Clinical Psychobiology Branch, NIMH; Alcohol Intramural Research
 Program, National Institute of Alcohol Abuse and Alcoholism; Tufts University

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Section on Clinical Studies

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

1.5

PROFESSIONAL:

1.0

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Increasing doses of the benzodiazepine, diazepam, or placebo were administered to normal volunteers or drug-free alcoholic inpatients in a single-blind, cross-over study. Following each dose saccadic eye velocity, electroencephalographic power spectra, diazepam blood levels, plasma cortisol, growth hormone and prolactin were measured and self-ratings of anxiety and sedation were performed. After every other dose cognitive testing of memory and attention was performed. The effects of diazepam on these variables was quantified and diazepam dose response curves constructed. These dose response curves provide a measure of benzodiazepine receptor sensitivity in humans.

OTHER PROFESSIONAL PERSONNEL

W. Mendelson	Chief, Unit of Sleep Studies	CP, NIMH
M. Linnoila	Clinical Director	ALC, NIAAA
D. Greenblatt	Tufts University School of Medicine	

PROJECT DESCRIPTION

Diazepam is administered intravenously in doses of 4, 4, 8, 17.5, 35, and 70 $\mu\text{g}/\text{kg}$ at 15 minute intervals. After each dose measurement of a variety biological and psychological variables that are affected by benzodiazepines is made. These variables include: electroencephalographic power spectra (done in conjunction with Dr. Wallace Mendelson), memory and attention (done with Dr. Herbert Weingartner), velocity of saccadic eye movements (done with Drs. Victor Matsua and Georgia Chrousos of the National Eye Institute), growth hormone, cortisol, prolactin and self-ratings of anxiety and sedation.

MAJOR FINDINGS

Plasma cortisol, saccadic eye velocity, self-rated sedation and recent memory all significantly decrease during diazepam administration while growth hormone concentration is increased significantly in most, but not all, individuals. From this data we constructed diazepam dose-response curves. These dose-response curves provide an in vivo measure of BZ receptor sensitivity in humans. Furthermore, we found extremely high correlations between all these variables during diazepam administration suggesting that these disparate effects are all mediated through the same class of BZ receptors. Prolactin and self-ratings of anxiety did not show significant dose-dependent changes during diazepam administration.

In addition to studying BZ receptor sensitivity in normals we have also examined BZ receptor sensitivity in two chronic alcoholic subjects who had been alcohol and drug-free for one month at the time of the study. Both of these subjects showed a much greater sensitivity to diazepam than any of the normal controls.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND TO THE PROGRAM OF THE INSTITUTE

Benzodiazepines are the most commonly prescribed psychotropic drugs in the world. The recent identification of stereospecific receptors for BZs in the brain of both animals and humans raises several new and exciting avenues of research not only into the mechanism of action of these anxiolytic and anticonvulsant agents but also into the pathophysiology of anxiety itself. For example, studies in rats have demonstrated that stress decreases the sensitivity of brain BZ receptors. If a similar phenomena occurs in humans it may provide the etiological link between stress and mental illnesses such as the anxiety, depression and post-traumatic stress disorder. In order to study BZ receptor sensitivity in psychiatric illness it is first necessary to develop a valid and reliable measure of BZ receptor sensitivity in normal human subjects. This project has provided such a measure of BZ receptor sensitivity and our preliminary results suggest that BZ receptor sensitivity may be altered in alcoholism.

PROPOSED COURSE

Further studies of BZ receptor sensitivity in various psychiatric disorders including anxiety and depressive disorders, as well as studies of the ability of various agents such as Ro-15-1788, a pure BZ receptor antagonist, and caffeine to block diazepam's actions in normals are planned. We also plan to study the effects of stress on BZ receptor sensitivity.

PUBLICATIONS

Hommer, D. W., Wolkowitz, D. M., Chrousos, G. A., Matsuo, V., Goldstein, D., and Weingartner, H.: Benzodiazepine receptor sensitivity in humans. Presented as New Research at APA Annual Meeting, Los Angeles, CA, 5-11 May 1984.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02188-01 NS

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (60 characters or less. Title must fit on one line between the borders.)

Biological Studies of Borderline Personality Disorder

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Rex William Cowdry Clinical Director NIMH

Others: D.L. Gardner	Staff Psychiatrist	NS, NIMH
K. O'Leary	Social Worker (Research)	OD, DIRP, NIMH
E. Turner	Social Worker (Research)	OD, DIRP, NIMH
R.L. Post	Chief	BP, NIMH
C. Kellner	Medical Staff Fellow	BP, NIMH
R. Coppola	Senior Engineer	LPP, NIMH

COOPERATING UNITS (if any)

Office of the Director, Division of Intramural Research Programs, NIMH; Biological Psychiatry Branch, NIMH; Laboratory of Psychology and Psychopathology, NIMH

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Section on Clinical Studies

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

1.8

PROFESSIONAL:

1.4

OTHER:

0.4

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Patients with borderline personality disorder and rejection-sensitive dysphoria participated in a program of clinical and biological evaluation and psychopharmacologic treatment. In addition to having labile moods and behavioral dyscontrol, these individuals were found to have a high incidence of psychosensory symptoms similar to those seen in complex partial seizures. There was a high incidence of non-specific electroencephalographic (EEG) abnormalities. One quarter of our patients had gross structural abnormalities on computerized axial tomography (CT) scans. Procaine infusions frequently produced dysphorias similar to those occurring naturally, and activated a high frequency band of EEG activity over the temporal lobes.

Group therapy employing small, diagnostically homogenous groups may have significant value in the treatment of borderline personality disorder.

A year-long, double-blind, placebo-controlled cross-over study of the effects of medication on mood and dyscontrol behavior suggested that alprazolam (a triazolo-benzodiazepine) may disinhibit these individuals, resulting in serious behavioral dyscontrol; that low-dose trifluoperazine (an antipsychotic), is not consistently different from placebo; that carbamazepine (an anticonvulsant) significantly decreases dyscontrol and produces improvement in physician (but not patient) ratings; and that tranylcypromine (a monoamine oxidase inhibitor with antidepressant actions) produced significant global improvement in mood and had variable effects on behavioral dyscontrol.

PROJECT DESCRIPTION

Rejection-sensitive or hysteroid dysphoria is a poorly understood syndrome occurring in many individuals with a diagnosis of borderline personality disorder. This syndrome, described by Klein and others, is characterized by the rapid onset of a dysphoric mood (sometimes characterized more specifically by depression, anxiety, or rage) following an actual, threatened, or imagined rejection. Behavioral dyscontrol is not uncommon, involving violence, direct injury to self, or overdosage with sedating medications. This disorder accounts for a significant number of admissions to short-term psychiatric units, and is one of the more difficult disorders treated in long-term outpatient psychotherapy.

There are a number of theories about the etiology of the borderline personality in general, and rejection-sensitive dysphoria in particular, most emphasizing developmental psychodynamics. Pathological parent-child interactions, particularly involving a failure of empathy during the symbiotic period or a failure of the separation-individuation process have been described as etiologic factors. Kernberg, while emphasizing the developmental abnormalities of this disorder, speculates that constitutional factors, such as an excess of aggressive drive, contribute to its development. However, very little research has been done to validate these hypotheses, or to explore possible biological mechanisms, such as abnormalities in limbic system functioning.

This project consist of two parts: (1) a clinical and biological evaluation, including symptomatology, developmental variables, neurophysiological function (EEG's and procaine-activated EEG's), and central nervous system structure (CT scans), and (2) trials of psychopharmacologic agents.

MAJOR FINDINGSPhenomenology

A close examination of the phenomenology of the dysphoric episodes which the borderline patient experiences reveals that these episodes are commonly precipitated by specific emotional stimuli (such as perceived rejection) and are generally relatively brief, although if the perceived stimuli continue they may develop into prolonged regression. The symptomatology is distinctive, usually involving disturbed mood (particularly intense emptiness, depression, rage or fear), and often having associated disturbances of thought processes (brief psychoses), of perception and cognition (usually distortions, hallucinations, depersonalization, derealization, deja vu), and of behavior (assault, overdoses, wrist cutting). Initial evaluation of data from our first 20 patients suggest that the incidence of psychosensory symptoms is high in individuals with borderline personality disorder, in many cases approaching the incidence in complex partial seizure patients.

Electroencephalographic (EEG) Studies

The symptomatology shows a striking overlap with that of complex partial seizures (CPS). The behavior is similar to that described as episodic dyscontrol (ED). Since both CPS and ED have associated electroencephalographic

abnormalities probably related to dysfunction of limbic or diencephalic structures, we hypothesize that the borderline syndrome also has a distinctive neurophysiological substrate involving limbic and diencephalic dysfunction.

EEG studies on our first 20 patients suggest that there is a relatively high incidence of non-specific EEG abnormalities, sometimes lateralized to the left or right temporal regions. Routine EEG's from age-matched controls and further EEG's from patients are needed to explore these differences in minor EEG abnormalities and correlate differences with subsequent medication response in the borderline group.

Procaine-Activated EEG's

Procaine-activated EEG's have been obtained in 13 patients to date, in collaboration with Drs. Robert Post and Charles Kellner (BPP) and Dr. Richard Coppola (LPP). One patient was not studied because of paroxysmal discharges on her routine EEG. All subjects had noteworthy reactions to procaine. Three showed little overt dysphoria, but two of these reported an increased wish for death coupled with a sense of calm. One became transiently paranoid (a state she also experiences spontaneously). One showed a euphoric response. Six subjects showed dramatic transient dysphoria, sometimes coupled with marked fear and a sense of impending death. One subject reported the occurrence of bloody mental images and another reported olfactory hallucinations. The majority of these subjects reported that the experience was quite similar to their spontaneously occurring dysphorias, except that it subsided rapidly. The most noteworthy features of the response were its dose-related nature, the degree to which it reproduced naturally occurring states, the rapidity with which it subsided (in contrast to the frequently prolonged natural course), and the efficacy of intravenous diazepam in treating the dysphoria when the dysphoria lasted more than a few minutes. In addition, a group of age-matched controls is being studied in order to demonstrate that the response seen in borderline individuals differs from that seen in individuals without a psychiatric history. To date, eight normal volunteers have been studied. None have experienced the marked dysphoria seen in the patients with borderline personality disorder, suggesting some specificity in the clinical response.

During the procaine infusion, EEG's have been recorded using both a special bilateral temporal montage developed by collaborators in Toronto, Canada, and a 16 channel left-hemisphere montage developed by Dr. Richard Coppola (LPP). The spectral analyses of both these EEG recordings demonstrate that procaine activates a high frequency (40-50 Hz) EEG band in the anterior and mid-temporal lobe regions, and suggest that the activation may occur most prominently in patients with rejection-sensitive dysphoria and behavioral dyscontrol. Preliminary analysis suggests a modest association between this high frequency activation and a therapeutic response to carbamazepine.

Computerized Tomography (CT) of the Cerebrum

CT scans of the cerebrum have been obtained in 19 patients for clinical indications such as severe headaches. Marked abnormalities were noted in five of these (Arnold-Chiari Malformation, focal atrophy of the left superior temporal lobe, generalized fronto-parietal sulcal effacement, hydrocephalis with

basilar and left temporal lobe calcifications due to tuberculosis, and marked asymmetry of the lateral ventricles). The most noteworthy aspects of the study to date are the marked pathological changes observed on the CT scan and the wide variety of sites involved in the lesions observed. These findings suggest non-specific contributions of gross central nervous system structural abnormalities to the occurrence of dysphoria and dyscontrol.

Treatment: Group Therapy

A pilot study of the use of group therapy in the treatment of these disorders was conducted by Kathleen O'Leary and Edward Turner. Most group therapists believe that individuals with borderline personality disorder do relatively poorly in group therapy. Our previous experience with homogeneous group therapy in bipolar affective disorder suggested major benefits from group therapy where all group members have the same disorder. Our experience with a small group of individuals who were program participants and were in concurrent individual psychotherapy suggests that homogeneous group treatment can be a valuable adjunctive treatment modality. Individual therapists, study investigators, and group therapists observed gains in social interaction, a sharing of behavioral methods found to help in dysphoric periods, and a decrease in members' perceived isolation over the course of the group. The majority of members themselves rated the experience as a positive and enriching one. From this pilot study and the perceptions and recorded responses of members, we are able to deduce which aspects of group therapy seem particularly well-suited to this population.

Treatment: Trials of psychopharmacologic agents

The usefulness of medication in the treatment of this disorder is highly controversial and has been minimally researched in controlled trials. Sixteen of the patients evaluated in the first part of this project entered a double-blind, placebo-controlled cross-over trial of four medications: alprazolam, a triazolobenzodiazepine; carbamazepine, an anticonvulsant; tranylcypromine, a monoamine oxidase inhibitor; and trifluoperazine, an antipsychotic. Each medication trial and the placebo trial consisted of a two-week dosage adjustment period, a four-week steady dosage treatment period, and a two-week period of dosage reduction and drug elimination.

Slight positive effects of placebo were seen in patient self-ratings; no effects were seen in blind observer ratings.

Alprazolam had positive effects in two patients, but was associated with serious episodes of behavioral dyscontrol in seven of the twelve patients treated with this medication, an incidence significantly higher than that on placebo. This increased incidence of dyscontrol on alprazolam is probably similar to the disinhibition observed with other benzodiazepines and with alcohol. Carbamazepine showed variable results in patient self-ratings, but was rated as significantly better than placebo by blind observers. Part of this positive rating was due to beneficial effects on mood, but the most striking finding was the total absence of serious episodes of dyscontrol and the significant reduction in day-to-day dyscontrol. The discrepancy between patient and physician ratings appears to be a result of the different value placed on mood as opposed to behavior. The subtle antidepressant and antianxiety effects, and

the marked antidyscontrol effects were not highly valued by the patients, who placed significant weight on clear-cut improvement in mood.

Low doses of trifluoperazine, the antipsychotic agent, had only moderate antianxiety effects. No differences from placebo were observed on other measures.

The monoamine oxidase (MAO) inhibitor, tranylcypromine, was the only agent rated significantly better than placebo by both patients and blind physician raters. Most patients experienced some degree of antidepressant and antianxiety effect, and some improvement in energy. However, serious dyscontrol was not significantly improved on this drug, in contrast to carbamazepine.

Improvement on carbamazepine was not correlated with improvement on tranylcypromine, suggesting that these agents may have different mechanisms of action and different clinical indications. The next phase of data analysis will focus on predictors of clinical response, in particular, whether major affective disorder symptoms predict antidepressant responses to the antidepressant tranylcypromine, and whether psychosensory symptoms or responses to procaine predict carbamazepine response.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND TO THE PROGRAM OF THE INSTITUTE

Rejection-sensitive dysphoria and borderline personality disorder are common disorders, particularly in the young adult population. They account for a significant number of short-term psychiatric hospitalizations and are frequently associated with major, often life-threatening overdoses, with self-mutilation, and with episodes of violence. The etiology of these disorders is a matter of great controversy and limited data, as is the role of medication in the treatment of these individuals.

The evaluation phase of this study provides tentative support for a theory of these disorders which emphasizes an interaction between developmental traumata and biological predisposition. If further studies confirm the association between low threshold for dysphoria and dyscontrol on the one hand and procaine induced high frequency EEG activity over the temporal lobe on the other, the link between limbic system abnormalities and labile mood and impulsive behavior is strengthened. Specific pharmacologic strategies for altering the responsivity of limbic system structures may ameliorate the dysphorias, may lessen the likelihood of dyscontrol, and may enhance the usefulness of psychotherapy in this disorder.

As previously noted, there is little evidence from controlled trials regarding the usefulness of medication in this disorder. The finding of significant clinical benefits from both an anticonvulsant and an antidepressant is important in itself, but suggests the value of further biomedical research to elucidate possible links between the biological underpinnings of this disorder and the biological mechanisms involved in mood disorders and in epileptic or epileptoid disorders.

PROPOSED COURSE

Further clinical, developmental, and biological data are needed from a larger number of patients. In particular, the procaine EEG studies will be expanded to draw firm conclusions about the relationship of procaine activation to diagnostic subgroups, clinical symptomatology, and drug responses. The procaine activation technique, coupled with higher resolution positron-emission tomography (PET), may permit visualization of brain structures involved in dysphoric states. Finally, further analysis of our current data may help identify pre-treatment predictors of response to medication, which would provide a controlled empirical base for decisions which are currently highly subjective.

PUBLICATIONS

None

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00117-09 NS

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Alpha-Adrenergic and Prostaglandin Receptors in Human Blood Elements

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: M. S. Kafka Physiologist NS, NIMH

Others: J. Nurnberger Psychiatrist BP, NIMH
 A. Roy Psychiatrist NS, NIMH
 T. Uhde Psychiatrist BP, NIMH
 R. Polinsky Neurologist CN, NINCDS

COOPERATING UNITS (if any)

Section on Clinical Studies, NS; Clinical Neuropharmacology, NINCDS; Unit on Anxiety and Affective Disorders, BP; Section on Psychogenetics, BP.

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Section on Molecular Pharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

0.7

PROFESSIONAL:

0.7

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Alpha-Adrenergic receptors and prostaglandin receptors are being studied in human blood cell preparations. The ability of adrenergic agonists to inhibit adenylate cyclase is used as a measure of the alpha₂-receptors' biological function. Binding of tritiated alpha-adrenergic receptor antagonists to membrane receptors on platelets is being measured. These correlative measures are used to assess alpha-adrenergic physiological function in normal individuals, patients with various psychiatric disorders, and patients receiving different psychopharmacologic agents.

Project Description:Objectives

- (1) To study alterations in α -adrenergic receptor number and function in psychiatric diseases and other pathological conditions characterized by altered adrenergic transmission. To measure changes in receptor number and function with different treatment modalities.
- (2) To provide an experimental model for the study of drug-induced or physiologically-induced changes in central receptor sensitivity in man.
- (3) To understand the cellular events connecting receptors, occupied by their agonists, with the activity of the adenylate cyclase enzyme complex and subsequent physiological events.

Methods Employed:

To measure α -adrenergic receptors, human platelets are prepared from a fresh blood sample. An aliquot of the platelets is washed and resuspended for measurement of cyclic AMP production. The remainder of the platelets are homogenized to yield a membrane preparation which is washed and resuspended for the measurement of tritiated α -adrenergic antagonist binding.

A method was worked out to measure adenylate cyclase activity in platelet lysates. With it, the prostaglandin E_1 stimulation, as well as the norepinephrine (NE) inhibition of cyclase activity could be measured and the EC_{50} (or IC_{50}) and B_{max} values derived.

An improved method for the binding of 3H -dihydroergocryptine to platelets was established. A saturation curve run on each subject's blood yielded an apparent affinity (K_D) and a B_{max} (Scatchard analysis) for binding to the platelet α_2 -receptor.

Major Findings:

- 1) In patients with orthostatic hypotension, the platelet findings differed with orthostatic hypotension of differing etiologies. Platelets from patients with idiopathic orthostatic hypotension or multiple system atrophy had more α_2 -receptors than platelets from control subjects. Platelets from patients with idiopathic orthostatic hypotension, but not multiple system atrophy, produced less PGE_1 -stimulated cAMP. Platelets from patients with sympathotonic orthostatic hypotension were similar to platelets from controls in both measures. The percent NE-inhibition of cAMP production was similar in platelets from orthostatic hypotensive patients and controls.
- 2) 3H -dihydroergocryptine binding to platelets from depressed patients was increased compared with platelets from control subjects. PGE_1 -stimulated cAMP production and NE-inhibition of PGE_1 -stimulated cAMP production were decreased in platelets from depressed patients. Receptor binding and functional responsiveness are dissociated in these patients, suggesting

the possibility that increased binding is secondary to a defect in α_2 -receptor responsiveness. Perhaps the increase in α_2 -receptors is due to uncoupling of the receptors from the adenylate cyclase complex. Alternatively, the increased number of α_2 -receptors may be secondary to decreased circulating NE. As the plasma NE concentration was not different in depressed patients and normal subjects, the second possibility may be less likely.

The number of binding sites, in any case, may not reflect the level of responsiveness of the α_2 -receptor. If neurons share with platelets the changes observed in depressed patients, it is possible that PGE₁-stimulation of cAMP and the functional responsiveness of the α_2 -adrenergic receptor are decreased in depressed patients.

- 3) In a small group of obsessive-compulsive patients (n=8), platelets had increased binding to α_2 -receptors and diminished PGE₁-stimulated cAMP production, as well as blunted NE-inhibition of PGE₁-stimulated cAMP production. The sample size was too small for a meaningful statistical comparison with controls, but the data were similar to platelet findings in panic/agoraphobic and in depressed patients, both of which groups were significantly different from controls. The data suggest that other tricyclic-responsive groups may share with patients with affective disorders a reduced responsiveness of the α_2 -receptor. The increase in binding to α_2 -receptors may be compensatory to this deficit, as discussed above. Perhaps tricyclic antidepressants increase the efficiency of α_2 -receptor coupling to the cAMP complex, compensating for the defect. It is possible that such compensation plays a role in the therapeutic effects of antidepressants.

Significance to Biomedical Research and to the Program of the Institute

The results obtained from these experiments are directly applicable to an assessment of receptor function in human disease states, and to monitoring the physiological effects of various drug treatments. If peripheral α_2 -adrenergic receptors are related to, or can serve as models for the central nervous system α_2 -receptors, valuable information about the function of these central receptors in psychiatric diseases may be obtained.

It is possible that α_2 -adrenergic receptor function in central and peripheral nervous systems is similar to that measured in platelets. Different platelet defects accompany orthostatic hypotension of different etiology, suggesting the usefulness of the platelet measure in studying alterations in hypotension with treatment. For example, platelets in idiopathic and multiple system atrophy hypotension differ in their defects, while in sympathotonic orthostatic hypotension, which is not characterized by peripheral blood vessel or baroreceptor defects, the platelet measures are like control. Longitudinal measurement of platelet α_2 -function might indicate which parts of the sympathetic nervous system are normalizing with a given treatment.

In depressed patients, and perhaps in patients with obsessive-compulsive disorder and panic-agoraphobic disorder, there may be a defect in α_2 -receptor coupling to the cAMP complex. The efficacy of antidepressants in correcting the defect might provide a means of evaluating the efficacy of that drug in treating the particular patient. The comparison of α_2 -receptor number during treatment, if increased α_2 -receptor number is a secondary concomitant of receptor uncoupling, might provide a relatively simple means of monitoring treatment efficacy, especially if a single B_{max} value for binding to the α_2 -receptor in blood could be shown adequate for use in monitoring treatment.

Proposed Course

To continue the examination of mechanisms of information transfer between α_2 -receptors and the adenylate cyclase complex. To measure in greater detail α_2 -adrenergic receptor function in platelets from patients with schizophrenia and affective illness. To investigate whether any changes measured are trait- or state-dependent, whether they are correlates of unipolar or bipolar illness, and whether they may be altered by some treatment modalities.

Publications

- 1) Siever, L.J., Kaye, W.H., Jimerson, D.C., Kafka, M.S., Lake, C.R., Targum, S., and Murphy, D.L.: "Abnormalities in the primary affective disorders compared to other tricyclic-responsive disorders". Psychopharmac Bull. 19: 435-436, 1983.
- 2) Kafka, M.S., Polinsky, R.J., Williams, A., Kopin, I.J., Lake, C.R., Ebert, M.H., and Tokola, N.S.: "Alpha-adrenergic receptors in orthostatic hypotension syndromes". Neurology, in press, 1984.
- 3) Siever, L.J., Uhde, T.W., Jimerson, D.C., Kafka, M.S., Lake, C.R., Targum, S., and Murphy, D.L.: "Clinical studies of monoamine receptors in affective disorders and receptor changes with antidepressant treatment. In Progress in Neuro-Psychopharmacology and Biological Psychiatry 7: 249-261, 1983.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00159-05 NS

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neurotransmitters Receptors in the Nervous System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: M.S. Kafka

Physiologist

NS, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Section on Molecular Pharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

0.3

PROFESSIONAL:

0.3

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Rat brain neurotransmitter receptors are being studied. There are circadian rhythms in neurotransmitter receptors in relatively discrete brain regions, as well as in whole "forebrain." In cerebral cortical adrenergic receptors there are circadian rhythms which are related to circadian rhythms in a functional response to receptor activation, such as the production of cyclic AMP (cAMP).

Project Description:Objectives:

- (1) To develop methods to measure the presence of neurotransmitter receptors in the nervous system.
- (2) To measure whether alterations in receptor number accompany alterations in function in the central nervous system.
- (3) To assess whether there are rhythms in functionally significant responses to receptor activation.

Methods Employed:

The specific binding of tritiated ligands to membranes prepared from rat brains is used to measure rhythmic changes in receptor binding in rats sacrificed at intervals over a 24-hour period. The α_1 -receptor is measured by the binding of ^3H -WB4101 or ^3H -prazosin; the β -adrenergic receptor, by ^3H -dihydroalprenolol; the muscarinic acetylcholine receptor by ^3H -QNB; the benzodiazepine receptor by ^3H -flunitrazepam; and the α_2 -receptor by ^3H -para-aminoclonidine. Methods to measure binding in very small tissue samples were devised.

Major Findings:

- (1) There are circadian rhythms in the α_1 -adrenergic receptor in most discrete brain regions measured. The peaks are usually in the dark part of the day when rats are active.
- (2) There is a circadian rhythm in arginine vasopressin in the suprachiasmatic nuclei (SCN). As arginine vasopressin is present in interneurons in this region, it may have a functional role in the generation or maintenance of the pacemaking function of the SCN.

Significance to Biomedical Research and the Program of the Institute:

Previous work documented the existence of circadian rhythms in neurotransmitter receptor density. These rhythms changed with chronic psychoactive drug administration. The new studies examine whether circadian rhythms in receptors are functionally significant. In vitro, the circadian rhythm in the number of α_1 - and β -adrenergic receptors stimulated by NE can regulate the circadian rhythm in cAMP production. Perhaps the number of adrenergic receptors stimulated by NE modulates the magnitude of cAMP production in situ. As the intraneuronal cAMP concentration is thought to act as a second messenger, regulating the phosphorylation and activation of cellular proteins, circadian rhythmic changes in neuronal cAMP production could have a profound effect on neuronal activity and neuronal transmission across the day.

Proposed Course:

Whether in situ there are circadian rhythms in neurotransmitter turnover,

receptors, and cAMP concentration, and, if so, what their relationships are, is being investigated in small brain regions. The relationship between rat brain circadian receptor rhythms and rat electroencephalographic patterns is being investigated. Receptor rhythms with multiple periods of sleep deprivation are being examined.

Publications:

- 1) Kafka, M.S.: "Central nervous system control of mammalian circadian rhythms. Introduction". Fed. Proc. 42: 2782, 1983.
- 2) Kafka, M.S., Wirz-Justice, A., Naber, D., Moore, R.Y, and Benedito, M.A.: "Circadian rhythms in rat brain neurotransmitter receptors". Fed. Proc. 42: 2796-2801, 1983.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00179-03 NS

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Morphological and Functional Aspects of Peptides in Mammalian Brain

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	L. Skirboll	Pharmacologist	NS, NIMH
	D.W. Hommer	Staff Psychiatrist	NS, NIMH
Others:	E. Mezey	Visiting Associate	LCB, NIMD
	J. Kiss	Visiting Associate	LCB, NIMH
	S. McLean	Staff Fellow	NS, NIMH
	C.P. Pert	Pharmacologist	NS, NIMH

COOPERATING UNITS (if any)

Section on Brain Biochemistry, NS, NIMH; Laboratory of Cell Biology, NIMH.

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Section on Molecular Pharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

2.0

PROFESSIONAL:

1.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Using immunohistochemical techniques in combination with retrograde tracing, we have extended our earlier findings in the paraventricular n. (PVN) to show that adrenalectomy (ADX) increases vasopressin (VP) immunostaining in the PVN and under these conditions VP and corticotropin releasing factor (CRF) coexist in single neurons in the brain. In addition, treatment of animals with an inhibitor which reduces brain epinephrine concentrations increased CRF staining suggesting that the innervating transmitter epinephrine may play a role in peptide expression. In addition we have established that it is an epinephrine-neuropeptide Y coexistent projection which innervates CRF neurons in the PVN. Finally, in addition to VP, in some cases cholecystokinin (CCK) coexists with CRF in the PVN. In a second series of studies we have extended our radioimmunochemical studies on the distribution of substance P and leucine-enkephalin in the rat brain. We find that these two putative transmitters now relate very well except in a few specific brain nuclei.

Project Description:

Objectives

The remarkable number of peptide neurotransmitters in the mammalian central nervous system has made it important to identify neuron populations on the basis of their chemical content. Isolation of neuropeptides and subsequent production of antisera to these compounds has led to the visualization of one and sometimes more transmitter(s) in a single neuron. This laboratory has been involved in the morphological and functional aspects of peptide transmitters in mammalian brain. Using immunohistochemical and electrophysiological techniques we have primarily explored the phenomena of coexistence (i.e., more than one putative transmitter substance in a single neuron). Immunocytochemical studies have been extended by using retrograde tracing techniques in which pathways can be traced on the basis of chemical transmitter.

Methods Employed

During the past year our laboratory has been using immunocytochemical techniques to further elucidate transmitter specific pathways using a combination of immunocytochemistry and retrograde tracing of fluorescent dyes. In general, animals are first treated with colchicine to block axonal transport of peptide and thus accumulate antigen in the cell body. This is followed by tissue preparation and incubation with antisera conjugated to a fluorescence molecule to allow visualization of transmitter-specific fluorescence of single cells under the fluorescence microscope. In some cases, the immunoperoxidase technique was employed. Studies in which peptide pathways were being explored were preceded by dye injected into proposed nerve terminal areas. The dye was taken up into axons and transported back into the cells of origin and visualized in the fluorescence scope. Once the dye is viewed, these same sections can subsequently be stained for immunocytochemical procedures as described above, thus permitting transmitter-specific mapping of neuroprojections.

Finally, since fluorescence or peroxidase-antiperoxidase (PAP) immunohistochemistry provide morphological detail but do not allow an easy assessment of peptide distribution (as they require a microscope with its narrow field of view), we have recently developed a radioimmunohistochemical technique. This method uses a radiolabeled antibody that allows us to visualize the antibody-antigen complex using autoradiography. Cryostat-cut sections are slide-mounted and then incubated with 1^0 antibody. Sixteen to 36 hours later the sections are rinsed and the ^{125}I -labelled secondary antibody, made against the 1^0 antibody, is applied. The slides are then opposed to LKB Ultrafilm or dipped in nuclear back emulsion.

Major Findings

As an extension of last year's work our interest in the hypothalamus and its involvement in several vegetative functions through which it integrates hypothalamic endocrine and autonomic responses to visceral stimuli was continued. There are several important transmitter systems in the paraventricular nucleus (PVN) including vasopressin (VP), corticotropin releasing factor (CRF), cholecystokinin (CCK) as well as innervating systems including adrenalin (A) and neuropeptide Y (NPY). We began a study to examine the morphological and physiological interactions between these systems. The immunoperoxidase technique was used to study the effects of adrenalectomy (ADX) on VP immunoreactivity in the PVN. It had been shown by others that ADX increased VP immunoreactivity in the median eminence (ME) which could be blocked by glucocorticoid treatment. We found that ADX increased the number and intensity of VP neurons stained the parvocellular portion of the PVN that are known to project to the ME. In addition this increase in VP staining produced a portion of cells which stained for both VP and CRF. These data suggest that both VP and CRF may be involved in the regulation of ACTH release. The mechanism by which ADX induces an increase in VP staining is unclear but these findings do suggest that ADX may lead to an increase in the synthesis of a VP precursor.

In a second series of studies, we examined the role of innervating transmitters on immunostaining in the PVN. We showed that there is a dense epinephrine innervation of the parvocellular subdivision of the PVN. These cells are primarily the CRF containing neurons which project to the ME. PNMT is the final enzyme in the biosynthesis of the adrenaline epinephrine and has been demonstrated in the brain. To examine the functional epinephrine significance of such an innervation, we treated animals with an PNMT inhibitor. We found that depleting epinephrine innervation of the PVN significantly increased CRF staining in this nucleus. These results suggest that central catecholamines may effect ACTH release through their action on CRF cells, perhaps by inhibiting the synthesis or expression of CRF. In order to confirm the epinephrine innervation of this subnucleus, fluorescent retrograde dyes were injected into the PVN. Dye labelled cells were observed in the C₁ area of the medulla. These cells stained for both PNMT and NPY. These data confirmed that there is a NPY-epinephrine coexisting system projecting to the PVN.

Finally in more detailed study of these CRF containing neurons which we found that CCK and CRF coexist in a subpopulation of neurons in the PVN suggesting that CCK may also play a role in the regulation of ACTH release.

In summary we have established that CCK, VP and CRF coexist in the PVN in varying degrees and that functional modifications on systems which upon these neurons are capable of altering neurotransmitter expression.

Finally, using the radioimmunochemical (RIC) technique developed last year we compared the distribution of Substance P and leucine-enkephalin in the mid and forebrain areas of the rat. We found that this technique facilitates examination of whole brain sections without magnification allowing one to discern the pattern of distribution of immunoreactive sites throughout the

brain. We also found this method to be slightly more sensitive than indirect immunofluorescence. Differences in the distribution of Substance P and enkephalin were seen in several areas of the brain including cortex, septum hippocampus and substantia nigra.

Projected Course

Since the PVN is an important area in neuroendocrine functions, we plan to continue our work addressing primarily the functional aspects of the system. In particular with regard to the phenomenon of coexistence (more than one putative transmitter in a neuron) studies of this area may prove to be fruitful. Our findings that CRF, CCK and VP coexist under different conditions may provide information on how each of these substances may regulate ACTH release and perhaps more importantly how each of these substances may interact to alter neuroendocrine function. Of equal interest is the finding that NPY and epinephrine coexist in neurons projecting to this area. In preliminary studies we have used standard electrophysiological techniques (see Hommer and Skirboll MH-02180) to examine the effects of stress on single unit activity in the PVN. We plan to extend these studies to look at the effects of these putative transmitter substances on activity in the PVN and to studies which hope to explore changes in activity as a result of adrenalectomy and enzyme inhibition.

We hope to apply radioimmunochemistry to several problems. Comparisons of published autoradiographic maps of receptors and immunohistochemical maps of the ligands do not show a perfect concordance. We plan to match autoradiography of receptors to immunostaining in an effort to address this problem. Ultimately, our goal is to do receptor autoradiography and radioimmunochemistry in adjacent sections from the same brain, thus making a direct comparison of receptor and ligand distribution. Finally, quantification of immunocytochemistry has been an elusive goal. The use of radioimmunocytochemistry allows the use of computer-assisted programs for quantitative autoradiography to start the process of quantifying immunocytochemistry.

Significance to Biomedical Research

Identification of more and more peptide putative transmitters in brain has permitted elucidation of a more refined network in mammalian nervous tissue. The use of the immunohistochemical technique which allows the tracing of chemical specific pathways in brain is only limited by the ability to raise antisera to a specific antigen. Such techniques especially used in common with retrograde tracing procedures will continue to reveal more about functional transmitter pathways in brain.

In particular, the ACTH response to stress is thought to be regulated by CRF and this response is modified in depressed patients. Thus, we hope that our studies on the morphology and function will provide new information on the delicate balance which regulates this neuroendocrine system.

Publications

1. Hokfelt, T., Vincent, S., Dalsgaard, C.J., Skirboll, L., Johansson, O., Schultzeberg, M., Lindberg, J., Rosell, S., Pernow, B. and Jansco, G.: Substance P: Immunohistochemical aspects on its distribution in brain and periphery and its possible role as a co-transmitter. In Porter, R., Conner, M. and Harman, P. (Eds.), Substance P in the Nervous System, London, 1983, pp. 84-99.
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3. McLean, S., Skirboll, L.R. and Pert, C.B. Opiatergic projection from the bed nucleus to the habenula: ademonstration by novel radioimmunohistochemical method. Brain Res. 278: 255-257, 1983.
4. Hokfelt, T., Lundberg, J., Skirboll, L., Johansson, O., Schulzeberg, M. and Vincent, S.: Coexistence of classical transmitters and peptides in neurons. In Cuello, C. (Ed.), Co-transmission. London, Macmillian, 1984, pp. 865-880.
5. Holtman, J., Skirboll, L., Norman, W., Dretchen, K., Hokfelt, T. and Gillis, R.: Evidence for serotonin, Substance P, and TRH in neurons innervating the phrenic motor nucleus. J. Neuroscience 4: 1064-1071, 1984.
6. Mezey, E., Kiss, J. Skirboll, L., Goldstein, M. and Axelrod, J.: Depletion of hypothalamic adrenalin increases corticotropin releasing factor staining in he PVN of the hypothalamus. Nature 310: 140-141, 1984.
7. Skirboll, L.R., Hokfelt, T., Kuypers, H.G.J.M., Bentovoglio, M., Catsman-Berrevoets, C., Goldstein, M., Steinbusch, A., Verhofstad, A., Steinbusch, J., Jeffcoate, S., Phillipson, O., Dockray, G., Brownstein, M. and Norell, G.: A method for specific transmitter identification of retrogradely labeled neurons: immunocytochemistry combined with fluorescence tracing. Brain Research Reviews, in press, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
 NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
 Z01 MH 02177-02 NS

PERIOD COVERED
 October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
 Behavioral Functions of Neuropeptides

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)
 PI: J.N. Crawley Senior Staff Fellow NS, NIMH

Others

L.R. Skirboll	Staff Fellow	NS, NIMH
D.W. Hommer	Staff Psychiatrist	NS, NIMH
E. Mezey	Visiting Associate	LC, NIMH
J.Z. Kiss	Visiting Associate	LC, NIMH
M.J. Brownstein	Chief	LC, NIMH
S.M. Paul	Chief	NS, NIMH

COOPERATING UNITS (if any)
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LAB/BRANCH
 Clinical Neuroscience Branch

SECTION
 Section on Molecular Pharmacology

INSTITUTE AND LOCATION
 NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS: 2.0	PROFESSIONAL: 1.0	OTHER: 1.0
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CHECK APPROPRIATE BOX(ES)

<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A). The past decade has witnessed the discovery of thirty or more peptides localized in mammalian central neurons. Cholecystokinin (CCK) a gut peptide was recently found in microgram concentrations in cerebral cortex, limbic structures, and spinal cord. Cholecystokinin appears to coexist with dopamine in the mesolimbic pathway from ventral tegmentum to nucleus accumbens and olfactory tubercle. Behavioral techniques provide useful tools for elucidating the mechanism of neuromodulation of a known neurotransmitter by a peptide. Understanding of CCK modulation of dopamine may lead to the development of more specific and effective antipsychotic treatments.

Animal studies have focused on anatomical sites of co-existence of cholecystokinin and dopamine (DA). In the nucleus accumbens, CCK potentiated the behavioral effects of DA and the dopaminergic agonist, apomorphine (APO). CCK did not potentiate the behavioral effects of DA and APO when injected into the caudate nucleus, where CCK and DA do not coexist. Specific antagonists of CCK, e.g. proglumide, benzotript, and CCK antibody, blocked the ability of CCK to potentiate DA-induced behaviors. These results suggest that CCK acts to modulate dopaminergic function in the mesolimbic pathway.

B). Gastrointestinal CCK has profound effects on feeding and exploratory behaviors, which have been characterized as the induction of a syndrome of "satiety". We are tracing the sensory feedback pathway from the gastrointestinal CCK receptors to brain regions which mediate feeding and exploration in rats. Lesions of the paraventricular nucleus of the hypothalamus, which receives rostral projections from the nucleus tractus solitarius, abolished the behavioral effect of CCK. One mechanism by which CCK induces satiety appears to require a sensory feedback pathway: GI tract→vagus nerve→nucleus tractus solitarius→rostral fibers→paraventricular nucleus of the hypothalamus.

Objectives:

A). Investigation of the neuromodulatory role of cholecystokinin (CCK) on dopamine-mediated behaviors. B). Tracing the cholecystokinin feedback loop relaying sensory information on feeding and satiety from the gut to the brain.

Methods:

Stereotypy and locomotor activity, exploratory behaviors, food consumption measurements, in mice and rats. Aseptic stereotaxic implantation of indwelling cannulae into brain nuclei of rats. Aseptic stereotaxic electrolytic and knife cut lesions of brain nuclei and pathways of rats. Microinfusion of peptides and drugs into brain nuclei. Histological verification of lesions, cannulae placements, and injection sites.

Major Findings:

A). Cholecystokinin (CCK), 20 pg - 200 ng, potentiated dopamine-induced hyperlocomotion and apomorphine-induced stereotypy when injected directly into the nucleus accumbens of rats. CCK injected alone, without DA or APO had no effect on locomotion or stereotyped behaviors. CCK, 2 ng, shifted the dose-response curve for APO to the left, as has been shown in the ventral tegmental cell bodies by Drs. Skirboll and Hommer using neurophysiological techniques. The anatomical sites of injection of CCK which produced the potentiation of DA-induced hyperlocomotion correlated with the anatomical sites previously shown by Drs. Hokfelt, Skirboll, and Hommer, to contain terminals from ventral tegmental cell bodies in which CCK and DA co-exist. These data lead to the conclusion that CCK acts as a modulator of dopaminergic function in the mesolimbic pathway.

CCK, 40 pg - 400 ng, did not influence dopamine-induced hyperlocomotion or apomorphine-induced stereotypy when injected directly into the caudate nucleus of rats. This finding suggests that CCK modulates dopamine only in sites of CCK-DA co-existence, since CCK and DA are co localized in the same neurons in the mesolimbic pathway, but in separate neurons in the nigrostriatal pathway.

The ability of CCK to facilitate dopamine was blocked by three specific CCK antagonists: proglumide, 20 µg, benzotript, 10 µg, and rabbit antiserum, 1:5 dilution raised against CCK. In addition, the facilitation was observed with sulfated CCK but not with unsulfated CCK. These findings suggest that the ability of CCK to modulate dopaminergic function is specific for the sulfated form of the CCK octapeptide, and not a generalized effect of peptide administration.

B). CCK has been implicated as a signal for feeding "satiety". Peripheral CCK receptors in the digestive tract have been shown to mediate food consumption, reducing total food intake in fasted rats, mice, sheep, pigs, monkeys, and human. We have previously shown that a variety of

behaviors associated with the behavioral state of the satiety are mimicked by intraperitoneally injected CCK₈-sulfate. These behaviors include reduced exploration of a novel environment, reduced approaches to a novel object, reduced social interactions, and increased periods of behavioral inactivity in the corners of an open field environment. Studies this year have focused on the anatomical pathways mediating this effect.

We have previously shown that lesions of the vagus nerve, which carries sensory information from the gut to the hindbrain, abolished the behavioral effects of intraperitoneally administered CCK. We have also shown that lesions of the nucleus tractus solitarius, where the visceral afferents of the vagus nerve terminate, also abolish the behavioral effects of intraperitoneally administered CCK. Drs. Kiss and Mezey are neuroanatomists from the laboratory of Dr. Miklos Palkovits in Budapest, Hungary, who are working in the laboratory of Dr. Mike Brownstein at NIMH. As they are experts in lesioning techniques, Drs. Kiss and Mezey are collaborating with our laboratory on performing discrete lesions with the midbrain and forebrain, to determine the sequential synaptic route from the nucleus tractus solitarius to brain regions which may mediate the effects of gut CCK on feeding and exploratory behaviors.

Midbrain transections, which destroyed all rostral projections from the nucleus tractus solitarius, completely abolished both the feeding and exploratory effects of CCK (5 ug/kg i.p.). Rats with incomplete transections, as verified histologically, showed only partial blockage of the CCK-induced behavior changes. These data indicate that structures or pathways rostral to the NTS are required for behavioral effects of CCK, eliminating the possibility that the behavioral effects are mediated solely by sites located in the hindbrain.

Discrete bilateral lesions of the paraventricular nucleus of the hypothalamus (PVN) completely blocked the effects of CCK (5 ug/kg i.p.) on feeding, and partially blocked the effects of CCK on exploratory behavior. Control lesions 1 mm rostral to the PVN had no effect on the ability of CCK to reduce food consumption and exploration, ruling out the possibility that fibers of passage through the PVN might be responsible. These data lead to the suggestion that the PVN is one site mediating the satiety syndrome initiated by CCK in the gut.

A second approach for investigating the feedback pathway from gut to brain which mediates CCK-induced behaviors is the identification of the neurotransmitters at each of the synaptic sites shown to be involved. Indwelling cannulae were implanted into the vagus-nucleus tractus solitarius synapse, in the parvocellular subdivision of the NTS. CCK at doses of 1 ng, 10 ng, and 100 ng injected into the NTS did not mimic the actions of intraperitoneally administered CCK, showing that CCK itself, while present in the NTS, is not the relevant transmitter at this synapse. Carbachol, 0.5 ug, injected into the NTS, effectively mimicked behavioral effects of intraperitoneally administered CCK, suggesting that acetylcholine may be the relevant transmitter at this synapse.

Proposed Course of Project:

A). Several studies are in progress or planned to further investigate the mechanism of action of CCK in modulating dopaminergic function. Combinations of dopaminergic antagonists and CCK antagonists will be simultaneously administered and tested for their combined ability to block DA-induced hyperlocomotion in the nucleus accumbens. If a CCK antagonist acts additively with a DA antagonist, then lower doses of DA antagonists can be used in combination with CCK antagonists to block dopaminergic function. If true, this concept could be applied to the development of new antipsychotic drugs, consisting of lower doses of neuroleptics plus low doses of CCK antagonists, which would have a lowered potential for the development of tardive dyskinesia.

To further investigate the basic mechanisms by which peptides modulate catecholamines, studies are planned for other anatomical sites receiving projections from nuclei where a peptide and a transmitter co-exist. These include the prefrontal cortex and the olfactory tubercle, where CCK-DA terminals are seen projecting from the ventral tegmentum. A second system of coexistence involves the septum and prefrontal cortex, where terminals containing Substance P, corticotropin releasing factor, and acetylcholine, are received from the lateral dorsal tegmental nucleus (identified by Dr. David Jacobowitz).

B). Several studies are in progress or planned to further investigate the sensory feedback pathway from gut to brain which mediates the CCK-induced "satiety" syndrome. Limbic sites in addition to the PVN which receive projections from NTS, and have been implicated in feeding or exploratory behaviors, include the central nucleus of the amygdala and the bed nucleus of the stria terminalis. These sites will be lesioned and tested for their role in mediating the behavior effects of intraperitoneally administered CCK. We are particularly interested defining sites which are critical for the feeding effects but not the exploration effects, or which mediate exploratory actions without changing feeding action CCK.

Pharmacological studies of the neurotransmitters at the PVN site are in progress. Stereotaxic coordinates for cannulating the PVN have been determined. Two neurotransmitters, serotonin and neuropeptide Y, which have been localized at PVN, are being injected to test their ability to mimic or block the behavioral effects of CCK (5 ug/kg i.p.).

Significance to Biomedical Research:

Several neuropeptides are colocalized with known transmitters. Their discovery in the past few years destroyed the dogma of one-neuron-one-transmitter. The functional significance of coexisting neuromodulators is one of the major basic research questions in neuroscience today. The clinical significance of this research lies in the relationship of CCK to dopamine in the psychopathology of schizophrenia. Cholecystokinin and dopamine coexist in the mesolimbic pathway considered a major site of action of antipsychotics. CCK agonists and antagonists may mimic, potentiate, or augment the actions of a neuroleptic.

A). Development of antipsychotic treatments which combine low doses of a neuroleptic with a cholecystokinin antagonist would advance the treatment of schizophrenia by reducing the risk for tardive dyskinesias. B). Investigation of neuronanatomical and neuropharmacological mechanisms regulating feeding and satiety may provide new insights into the treatment of anorexia nervosa and bulimia.

Publications:

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4. Crawley, J.N. Divergent effects of cholecystokinin, bombesin, and lithium on rat exploratory behaviors. Peptides 4: 410-415, 1983.
5. Charlton, C.G. Miller, R.L., Crawley, J.N., Handelsmann, G.E. and O'Donohue, T.L. Secretin modulation of behavioral and physiological functions in the rat. Peptides 4: 739-742, 1983.
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7. Crawley, J.N., Kiss, J.Z. and Mezey, E. Bilateral midbrain transections block the behavioral effects of cholecystokinin on feeding and exploration in rat. Brain Research, in press.
8. Crawley, J.N. and Kiss, J.Z. Tracing the sensory pathway from gut to brain regions mediating the actions of cholecystokinin on feeding and exploration Annals of the New York Academy of Sciences, in press.
9. Crawley, J.N. Divergent effects of cholecystokinin, bombesin, and lithium on rat exploratory behaviors. Brain Res. Bull., 4: 405-410, 1983.
10. Crawley, J.N. and Schwaber, J.S. Nucleus tractus solitarius lesions block the behavioral actions of cholecystokinin. Peptides, 4: 743-747, 1983.

11. Crawley, J.N. and Schwaber, J.S. Abolition of the behavioral effects of cholecystokinin following bilateral radiofrequency lesions of the parvocellular subdivision of the nucleus tractus solitarius. Brain Res., 295: 289-299, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
 NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02178-02 NS

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neuropharmacology of Anxiety

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J. N. Crowley Senior Staff Fellow NS, NIMH
 J. R. Glowa Senior Staff Fellow NS, NIMH

Others: S. M. Paul Chief NS, NIMH
 P. Skolnick Pharmacologist LBC, NIAODK
 S. Maier Professor, Dept. of Psych. Univ. of CO

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LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Section on Molecular Pharmacology

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

1.5

PROFESSIONAL:

1.0

OTHER

0.5

CHECK APPROPRIATE BOX(ES)

(a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The discovery of brain recognition sites specific for benzodiazepines opened the field for identifying specific brain mechanisms mediating anxiety. Rodent and primate models of anxiety were applied to the analysis of the anxiety-producing properties of a series of β -carboline compounds previously shown to bind with high affinity to the brain benzodiazepine site. Sensitivity and specificity of the pharmacologically-induced "anxiety" response was investigated. In the conflict test, rhesus monkeys showed a significant decrease in punished and non-punished responding at doses of β -CCE as low as 50 ug/kg. In the learned helplessness model, rats treated with FG-7142 developed an inability to learn an escape task twenty four hours later, equivalent to the long-lasting effects of inescapable tailshock. These results suggest that the "active antagonists" of the brain benzodiazepine receptor induce a response which may be interpretable as stress-induced anxiety.

Objectives:

The recent discovery of benzodiazepine receptor antagonists led to intensive investigation of anxiogenic or anxiety-inducing properties of brain benzodiazepine receptor blockers. Our hypothesis rests on the postulated existence of an endogenous substance or substances which normally activate and/or inhibit the neuronal firing of cell groups containing benzodiazepine receptors. Pharmacological agents which block the receptor should then produce a response active but opposite to receptor agonists, just as isoproterenol stimulates and propranolol blocks β -adrenergic receptors, producing opposite cardiovascular effects. The objective of the project is the identification of anatomical sites and neuropharmacological mechanisms of action of anxiety-producing and anxiety-reducing drugs.

Methods Employed:

Punished responding for food in chair-adapted rhesus monkeys;
Learned helplessness test for ability to learn an escape task after a session of inescapable shock, or after treatment with drugs acting at the brain benzodiazepine binding site.

Major Findings:

The standard rat conflict test was modified for use with rhesus monkeys by Dr. John Glowa. Monkeys maintained at 70% of body weight were presented with a response task in which food reward was contingent on acceptance of low voltage electric shock. Diazepam effectively increased punished responding in a dose-related fashion. 3-Carboethoxy- β -carboline (β -CCE) effectively decreased punished responding in a dose-related fashion. Significant reductions in responding were found with extremely low doses of β -CCE, e.g. 50 ug/kg i.v. Non-punished responding was also decreased by β -CCE, which may be interpreted as indicating that the effects of β -CCE are generalized stress responses rather than specific responses to a task thought to represent anxiety.

The learned helplessness paradigm was modified by replacement of the session inescapable shock with a single injection of FG-7142, a β -carboline with a relatively long half-life in rodents. Administration of FG-7142 resulted in behavioral effect equivalent to a session of inescapable tailshock. Rats treated with FG-7142 failed to acquire an escape response twenty-four hours after treatment. Pretreatment of rats with the selective benzodiazepine receptor antagonist, Ro-15-1788, blocked the development of learned helplessness elicited by FG-7142. These findings suggest that "anxiety", induced pharmacologically by an active benzodiazepine receptor antagonist, may be causally related to the development of learned helplessness.

Proposed Course of the Project:

To determine the site of action of β -CCE in reducing punished responding in the monkey conflict test, the selective benzodiazepine receptor blocker, Ro-15-1788, will be administered prior to β -CCE injection and behavioral testing. Blockade of the β -CCE effects by Ro-15-1788 would support the

hypothesis that the brain benzodiazepine receptor specifically mediates the actions of β -CCE on reducing punished responding (Dr. Glowa).

Dr. Drugan will be joining our unit in October, 1984 as a postdoctoral fellow. He will investigate anatomical sites in the rodent brain which may mediate both anxiety-related and depression-related behaviors.

Significance to Biomedical Research:

Responses to benzodiazepine receptor antagonists in primates are being analyzed for their relevance to human anxiety disorders. Basic research into the functional significance of the brain benzodiazepine binding site may provide new approaches to the treatment of panic disorders and anxiety neuroses. Studies the anatomical and pharmacological sites critical to both the rat conflict test for anxiety and the rat learned helplessness model for depression are designed to provide information on the role of environmentally-induced anxiety in the development of human depression.

Publications:

1. Crawley, J.N., Skolnick, P. and Paul, S.M. Absence of intrinsic antagonist actions of benzodiazepine antagonists on a mouse exploratory model of anxiety. Neuropharmacology 23: 531-537, 1984.
2. Skolnick, P., Ninan, P., Insel, T., Crawley, J. and Paul, S. A novel chemically induced animal model of human anxiety. Psychopathology 17: 25-36, 1984.
3. Crawley, J.N., Ninan, P.T., Pickar, D., Chrousos, G.P., Skolnick, P. and Paul, S.M. Behavioral and physiological responses to benzodiazepine receptor antagonists. Psychopharmacology Bulletin, in press.
4. Crawley, J.N., Ninan, P.T., Pickar, D., Chrousos, G.P., Linnoila, M., Skolnick, P. and Paul, S.M. Neuropharmacological antagonism of the β -carboline-induced "anxiety" response in rhesus monkeys. Journal of Neuroscience, in press.
5. Ninan, P.T., Pickar, D., Schulte, H.M., Chrousos, G., Crawley, J., Skolnick, P. and Paul, S.M. Benzodiazepine/GABA receptor-mediated release of ACTH and β -endorphin in the primate. J. Clinical Endocrinology and Metabolism, in press.
6. Crawley, J.N. Exploratory behavior models of anxiety in mice. Neuroscience and Biobehavioral Reviews, in press.
7. Crawley, J.N., Blumstein, L.K. and Baldino, F. Anxiolytic-like properties of fominoben. Eur. J. Pharmacol. 97: 277-281, 1984.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02179-02 NS

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Hamster Separation Model of Depression

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J.N. Crawley Senior Staff Fellow NS, NIMH

Others: S.M. Paul Chief NS, NIMH
 R.L. Hauger Staff Psychiatrist NS, NIMH
 I. Angel Guest Researcher NS, NIMH
 A.J. Janowsky Staff Fellow NS, NIMH
 R. Labarca Visiting Fellow NS, NIMH

COOPERATING UNITS (if any)

Laboratory of Brain Evolution and Behavior, NIMH: Section on Preclinical Studies
 NSB, NIMH.

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Section on Molecular Pharmacology

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

1.5

PROFESSIONAL:

1.0

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A novel hamster separation model of depression is under development for analysis of central neurochemical changes during a behavioral state of separation which may be analogous to human depression. Separated male hamsters show increased body weight, decreased exploratory behaviors, and reduced social interactions. Analysis of the time course of development of the syndrome found that a minimum of one week of separation was required. The syndrome was completely reversed by re-pairing with the original female partner. The syndrome was only partially reversed by re-pairing with a new female partner, or with a male partner. Chronic treatment with a monoamine oxidase inhibitor, tranylcypromine, was found to completely reverse the syndrome beginning at fourteen days of treatment. Receptor binding assays revealed a significant decrease in number of binding sites for ³H-amphetamine in the brainstem of separated male hamsters, as compared to paired male hamsters, suggesting that the observed change in body weight during the separation period may reflect a change in central regulation of feeding or glucose utilization.

P. MacLean Chief
R.J. Weber Senior Staff Fellow

LBEB NIMH
NS, NIMH

Project Description:

Objectives:

Development and evaluation of a new rodent model for depression. Characterization of specific, reproducible, quantitative changes in behavior. Evaluation of pharmacological specificity of antidepressant treatments in reversing the behavioral changes. Investigation of the changes in brain receptor sensitivity and neurotransmitter function during the defined state of social separation in the Siberian dwarf hamster.

Methods Employed:

Breeding, pairing, and separating of dwarf hamsters; behavioral rating of exploratory and social behaviors; receptor binding assays.

Major Findings:

The psychopharmacology of depression remains a complex issue, requiring better animal models for testing hypotheses of neurochemical abnormalities. The two animal models in current use are the Wisconsin monkey separation paradigm and the Seligman learned helplessness syndrome. Primate studies have the disadvantage of small sample size and limitations on studies of brain chemistry. Learned helplessness is a paradigm employing inescapable foot shock, primarily modeling the long term effects of stress. We are attempting to develop a better rodent model, which has both the advantages of large sample size and access for neurochemical assays, and closer conceptual and behavioral analogies to human depression. *Phodopus sungorus* is a rare species of Siberian dwarf hamster. It is reported to have a social system of male-female pair bonding, which is unusual among rodents. Anecdotal reports suggested that separation of the members of a male-female pair resulted in a behavioral syndrome with analogies to human depression.

Our original studies demonstrated a significant increase in body weight, decrease in exploratory behaviors, and decrease in social interactions during the separation period. These changes were seen predominantly in the male hamsters, with females showing minor or non-significant changes. Three new behavioral studies have been completed. 1) The time course for the development of the syndrome has been determined as beginning at one week of separation, maximal by two weeks of separation, and persisting intact for at least four weeks of separation. 2) A series of pairings and separations has been completed to determine the specificity of the syndrome to an established male-female pair bond. Re-pairing with the original female reversed all of the behavioral changes. Re-pairing with an unfamiliar female did not significantly reverse any of the behavioral changes. Repairing with another

male partially reversed the deficits in exploration and social interactions. Thus, re-pairing with the original female was more effective than re-pairing with another female or with a male.

3) Genetic analysis of the heritability of a strong separation response was performed by testing the first generation offspring of males with strong separation responses and the first generation offspring of males with weak separation responses. Strength of response by a male parent was not found to be predictive of strength of response in a male offspring.

The second drug trial with an antidepressant agent in separated hamsters utilized the broad spectrum monoamine oxidase inhibitor, Parnate (Tranlylcypromine Sulfate, gift of Smith, Kline and French Company). Parnate, 10 mg/kg/day, s.c., reversed all of the behavioral effects of separation, beginning on day 14 of drug administration. Saline, 0.1 ml/day, s.c., for two weeks following cessation of Parnate administration, reinstated the behavioral effects of separation. These data show that an antidepressant drug treatment is effective in abolishing the separation syndrome.

Receptor binding assays were performed on brain regions from seven paired and eight separated male hamsters. No significant difference was found between the two groups on ^3H -imipramine binding (Dr. Itzhak Angel), ^3H -spiperone binding (Dr. Richard Hauger), ^3H -flunitrazepam binding (Dr. Steven Paul, Ms. MyDo Luu), or carbachol-stimulated ^3H -inositol-1-phosphate accumulation (Dr. Aaron Janowsky, Dr. Rodrigo Labarca). However, a significant decrease in ^3H -amphetamine binding ($p < .025$) was detected in the brainstem of separated male hamsters (Dr. Richard Hauger, Dr. Steven Paul, Ms. Bridget Giblin). This amphetamine binding site has previously been shown to be linked to feeding behaviors, and may represent a glucose-sensitive receptor involved in homeostatic regulation. Changes in receptor number at this site may be involved in the increased body weight observed in the separated male hamsters.

Proposed Course of the Project:

Further drug trials are planned to test the pharmacological selectivity of the hamster separation model for antidepressants. Several other monoamine oxidase inhibitors and tricyclic antidepressants will be tested, as well as other categories of drugs such as neuroleptics and anxiolytics.

A comprehensive analysis of catecholamines and metabolites, using high pressure liquid chromatographic techniques, is scheduled for the next large population of paired and separated hamsters (Dr. Markku Linnoila, NIAAA).

Immunological analysis of separated and paired hamsters is in progress to determine whether the separation syndrome involves dysfunctions of immune suppression responses (Dr. Rick Weber).

Significance to Biomedical Research and the Program of the Institute:

Phodopus sungorus is a rapidly breeding, easily maintained species. Its usefulness as a model for human depression will be determined over the next

two years. Such a model could be applied to the development of new classes of antidepressants. At the level of basic research, the model could provide a discrete population for testing current theories of neurochemical dysfunctions in depression.

Publications:

1. Crawley, J.N. Preliminary report of a new rodent separation model of depression. Psychopharmacology Bulletin 19:537-541, 1983.
2. Crawley, J.N. Evaluation of a proposed hamster separation model of depression. Psychiatry Research 11:35-47, 1984.
3. Crawley, J.N. Preliminary report of a new rodent separation model of depression, in Progress in Neuro-psychopharmacology and Biological Psychiatry, in press, 1984.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02180-02 NS

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Electrophysiological Studies
of Peptidergic and GABAergic Function in Mammalian Brain

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	D. Hommer	Staff Psychiatrist	NS, NIMH
	L. Skirboll	Staff Pharmacologist	NS, NIMH
Others:	S. M. Paul	Chief	NS, NIMH
	P. Skolnick	Pharmacologist	LBC, NIADDK
	J.N. Crawley	Pharmacologist	NS, NIMH

COOPERATING UNITS (if any)

Laboratory on Bioorganic Chemistry, NIADDK.

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Section on Molecular Pharmacology

INSTITUTE AND LOCATION

ADAMHA, NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

2.6

PROFESSIONAL:

2.0

OTHER:

0.6

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Using extracellular single unit recording techniques we have found that sulfated cholecystokinin (CCK) octapeptide when administered either systemically or iontophoretically potentiates the actions of dopamine (DA) on DA autoreceptors in the mesolimbic DA system. Furthermore, we found that all varieties of CCK-like peptides which bind to brain CCK receptors also potentiate DA in the ventral tegmental area (VTA) and medial substantia nigra (SN), but that those CCK-like peptides which do not bind to brain CCK receptor sites were ineffective in potentiating DA. We have also found that the putative CCK antagonists, proglumide and benzotript, weakly blocked CCK in proportion to their potency at central CCK receptors. The ability of CCK to potentiate DA only occurs in those midbrain regions where DA and CCK coexist.

We have also found that the anxiogenic benzodiazepine (BZ) receptor ligand, beta-carboline carboxylate ethyl ester (βCCE) increases the activity of neurons in the SN zona reticulata but has no effect on noradrenergic neurons in the locus coeruleus. Caffeine also mimics many of the effects of βCCE in the SN but its actions are not reversed by the specific BZ antagonist Ro-15-1788 as are those of βCCE. It appears that the substantia nigra zona reticulata is sensitive to the effects of anxiolytic and anxiogenic compounds, as well as anticonvulsant and proconvulsant drugs.

Project Description:Objectives:

Immunohistochemical studies have shown that there is a coexistence of dopamine (DA) and cholecystokinin (CCK) in a subpopulation of mesencephalic neurons which in the rat project primarily to limbic areas. Previously, we reported that these DA/CCK containing cells are excited by either systemically or iontophoretically administered CCK. This raises the question of the functional significance of DA/CCK coexistence (i.e., how does the peptide CCK and the classical catecholamine neurotransmitter, DA, interact to affect the activity of neurons).

We have previously shown that systemically administered CCK-8 sulfate increases the potency of DA and DA agonists at the DA autoreceptor in the A-10 and medial A-9 region of the rat midbrain. Our purpose in the series of experiments undertaken this year was to further characterize this effect and to determine if it occurs with other forms of CCK in addition to CCK-8 sulfate.

The substantia nigra (SN) zona reticulata (ZR) is a region which contains a high concentration of GABA and benzodiazepine (BZ) receptors. Recently, several groups have shown that microinjections of GABA agonists into the SN but not into adjacent mesencephalic areas, blocks electrically-induced seizures in rats. These microinjections also block the limbic after discharge following kindled seizures. This suggests that the SN may be an important region involved in propagation of seizure activity as well as in modulation of limbic system function. Since BZ can block seizures and alter behavior presumably mediated through limbic regions, the SN ZR represents an ideal location in which to study BZ actions using electrophysiological techniques.

We also examined the actions of β CCE, Ro-15-1788 and diazepam on the activity of identified neurons in the rat locus coeruleus (L.C.). The L.C. has figured prominently in a number of theories of the pathophysiology of anxiety. Thus the effects of new anxiogenic drugs such as β CCE on this structure are of considerable interest.

Methods Employed:

To investigate these questions we used extracellular single unit recording techniques in chloral hydrate anesthetized male albino rats. We used both systemic and iontophoretic administration of drugs and neurotransmitters

Major Findings:

As we have previously reported ceruletide and CCK-8 S_{04} both potentiate the effects of the DA agonist, apomorphine, on DA neurons in the medial SN. In addition to this we have also found that CCK-8 unsulfated and CCK-4 also possess a similar ability to potentiate apomorphine induced inhibition in the SN. The in rank order potencies are as follows: ceruletide, CCK8 S_{04} , CCK8 unsulfated and CCK-4. CCK3 was without effect. These potencies directly

parallel the affinity of these peptides for the brain CCK receptor. This suggests that potentiation of dopaminergic neurotransmission by CCK is a specific receptor mediated phenomena and probably of physiological relevance.

We have also found that all electrophysiologically active CCK peptides have the ability to potentiate DA only in region's of DA/CCK coexistence. This phenomenon occurs during both systemic administration and iontophoretic application of peptide and DA agonist.

These findings suggest that a possible function of CCK/DA coexistence is to specifically modulate the sensitivity of the DA neurons which comprise the mesolimbic and mesocortical DA projections thus providing a functional separation of limbic from striatal dopaminergic neurotransmission.

In addition to examining the effects of CCK agonists on nigral DA neurons we also have examined the ability of two putative CCK antagonists to block the effects of CCK. We found that proglumide when administered systemically could block CCK action on midbrain DA neurons only at very large doses of 80-160 mg/kg. Benzotript another putative CCK antagonist was effective at blocking CCK in doses of 20-60 mg/kg administered intravenously. These potencies of proglumide and benzotript parallel their affinity for the brain CCK receptor indicating that our electrophysiological technique provide a valid model for studying the pharmacological actions of CCK antagonists as well as agonists.

We have found that the β -carboline derivative β CCE, a compound which binds to the BZ receptor and causes severe anxiety in primates, potently excites neurons in the SN ZR. This excitation is reversed by the BZ antagonist Ro-15-1788, and is in contrast, to the actions of the "peripheral" BZ receptor ligand, Ro-5-4864, which although it also increases the activity of SN ZR neurons, could not be reversed by Ro-15-1788. We have also found that several different BZs potentiate the action of iontophoretically applied GABA on SN ZR neurons, and that the ability of these BZ's to potentiate the inhibitory action of GABA in the SN ZR positively correlated with the drugs potency as hypnotics but not with their potency in tests of their anti-conflict and anti-convulsant properties.

We also found that β CCE significantly attenuates GABA's inhibitory effects in the SN. This inhibition was readily reversed by the specific BZ antagonist Ro-15-1788. Caffeine, in doses which are similar to those consumed in several cups of coffee, also produced a significant attenuation of GABAergic inhibition of SN ZR neurons, however, this inhibition could not be blocked or reversed by Ro-15-1788.

In addition to examining the actions of BZ receptor agonists and antagonists in the SN we have also studied the effects of β CCE, diazepam and Ro-15-1788 in the locus coeruleus (L.C.) Diazepam produced a significant decrease in activity of noradrenergic neurons in the L.C. and the decrease could be reversed by either Ro-15-1788 or β CCE, however, β CCE was entirely without effect when administered alone. This is in marked contrast to β CCE's excitatory effects in the SN ZR and suggests that activation of L.C. is not essential for anxiety induced by agents acting on BZ receptors.

Projected Course:

We have now completed our planned investigation into CCK/DA interactions. Our efforts during the next year will be concentrated in two areas. We plan to continue studies of the effects of BZ and caffeine in SN ZR and extend these studies to the globus pallidus. One of our areas of concentration will be interaction between GABA/BZ systems, stress and stress related hormones.

We also plan to investigate the interaction of neuropeptide Y and adrenaline on CRF containing cells of the paraventricular nucleus of the hypothalamus. These two putative transmitters coexist in a system which appears to innervate CRF neurons which play a primary role in control of ACTH. These studies will subsequently look at the effects of stress and adrenalectomy on the firing of these neurons.

Significance to Biomedical Research and the Program of the Institute:

Although our initial controlled studies with CCK in schizophrenic patients have proved negative our findings that unsulfated CCK as well as sulfated CCK potentiates dopamine suggests that further clinical trials with unsulfated CCK could be a value. By using unsulfated CCK the dose of the drug could be increased without producing the severe gastrointestinal side effects which limit the usefulness of sulfated CCK.

On a more basic level the study of interactions between CCK and DA may provide a prototype for interaction between peptide/classical neurotransmitter coexisting systems.

Electrophysiological studies of BZ and GABA compliment ongoing biochemical and behavioral studies in these areas. The SN appears to be an important brain region in the modulation of seizures. Electrophysiological techniques are particularly well suited to further our understanding of how the SN performs this function. Understanding of how stress affects the GABA/BZ receptor systems should be of potentiate value in developing new pharmacological approaches to stress related diseases, both medical and psychiatric.

Publications:

1. Hommer, D.W. and Pert, A.: The actions of opiates in the rat substantia nigra: an electrophysiological analysis. Peptides, 4: 603-608, 1984.
2. Hommer, D.W. and Skirboll, L.: Cholecystokinin-like peptides potentiate apomorphine-induced inhibition neurons. Eur. J. Pharmacol., 91: 151-152, 1983.
3. Weissman, B.A., Cott, J., Hommer, D.W., Quirion, R., Paul, S.M. and Skolnick, P.: Pharmacological, electrophysiological, and neurochemical actions of Ro-5-4864 (4'chlorodiazepam). In Biggio, G. (Ed.): Benzodiazepine Recognition Site Ligands: Biochemistry and Pharmacology. New York, Raven Press, 1983, pp. 139-151.

4. Weissman, B.A., Cott, J., Hommer, D., Paul, S.M., and Skolnick, P.: Electrophysiological and pharmacological actions of the convulsant benzodiazepine Ro-5-4864. Eur. J. Pharmacol. 97: 257-263, 1984.
5. Weber, K.H., Kuhn, E.J., Boke-Kuhn, K., Lehr, E., Danneberg, P.B., Hommer, D.W., Paul, S.M., and Skolnick, P.: Pharmacological and Neurochemical Properties of 2,4-Diazepines with two annelated heterocycles ("Hetrzepines"). Eur. J. Pharmacol., in press, 1984.
6. Skirboll, L.R., Stoner, G.R., and Hommer, D.W.: Interactions between CCK and DA: an electrophysiological analysis. In Vanderhoeghen, J.J. and Crawley, J. (Eds.): Neuronal Cholecystokinin, New York Academy of Science, in press, 1984.
7. Crawley, J.N., Hommer, D.W., Skirboll, L.R.: Behavioral and neurophysiological evidence for a facilitatory interaction between co-existing transmitters: cholecystokinin and dopamine. Neurochemistry International in press, 1983.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00167-05 NS

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Biochemical Coding of Brain Pathways Revealed by Autoradiography

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

I: C. B. Pert Pharmacologist NS, NIMH

Others: R. B. Rothman Guest Researcher NS, NIMH

S. McLean Staff Fellow NS, NIMH

M. A. Herkenham Psychologist LN, NIMH

COOPERATING UNITS (if any)

Laboratory of Neuropsychology, NIMH

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Section on Brain Biochemistry

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

 (a) Human subjects (b) Human tissues (c) Neither (a1) Minors (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been incorporated into Z01 MH 02189-01 NS.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00169-04 NS

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Allosteric Receptor Modulation and Altered Sensitivity States

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: C. B. Pert Pharmacologist NS, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Section on Brain Biochemistry

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS

0

PROFESSIONAL

0

OTHER

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been incorporated into Z01 MH 02190-01 NS.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02182-02 NS

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Toward the Visualization of Opiate Receptors in Living Humans

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: C. B. Pert Pharmacologist NS, NIMH

Others: J. A. Danks Guest Researcher NS, NIMH
 M. A. Channing Physician NM, CC
 W. C. Eckelman Chief, Rad. Chem. Sec. NM, CC
 S. M. Larson Chief NM, CC
 T. R. Burke Pharmacologist LC, NIADDK
 K. C. Rice Pharmacologist LC, NIADDK

COOPERATING UNITS (if any)

Nuclear Medicine, Clinical Center, Laboratory of Chemistry, NIADDK

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Section on Brain Biochemistry

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

0.6

PROFESSIONAL:

0.6

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The distribution of positron emitting substances in brain can be followed by positron emission tomography (PET). We are developing ^{18}F -labeled high affinity opiate drugs to be injected into living humans for the visualization of opiate receptor patterns in vivo. It will be interesting to determine whether opiate receptor distribution patterns in cortex change as a function of attention and emotional states.

Project Description:Objectives:

To demonstrate gradients of opiate receptor density in the cortex of living humans. To examine whether differences in these gradients exist as a function of emotional state or attentional processes.

Methods Employed:

PET Scan--using newly developed ^{18}F -labeled opiate analogs. Autoradiography of rat brain slices.

Major Finding:

We managed to affix a fluoride moiety to naltrexone, a potent opiate antagonist without losing affinity for opiate receptors. This fluoro-opiate derivative is suitable for in vivo injections for visualizing receptors. We have visualized stereospecific, striking images of opiate receptors in the thalamus, basal ganglia and frontal cortex of a living baboon.

Significance to Biomedical Research and Program of the Institute:

The notion that alterations in mood are a function of oscillations in neurotransmitter receptor sensitivity is perhaps the most exciting new lead in attempting to understand the causes of mental illness. Other leads in this institute point to the relevance of cortical participation as a critical factor in psychiatric disease. The opiate receptor is the most well-studied of brain receptors and appears to be associated with the pleasure of fulfilled appetite.

Proposed Course:

Our new useful probe, [^3H]-3-acetylcyclofoxy, will be characterized thoroughly in rodents to fulfill requirements for human use. Human studies, first on normal controls, then psychiatric patients, should enable a rigorous test of theories of emotions which emphasize neuropeptide receptors.

Publications:

1. Rice, K.C., Konicki, P.E., Quirion, R., Burke, T.R. and Pert, C.B. Synthesis and pharmacological characterization of (\pm)-5,9 α -dimethyl-2-[2(4-fluorophenyl)ethyl]-2'-hydroxy-6,7 benzomorphan (Fluorophen). A ligand suitable for visualization of opiate receptors in vivo. J. of Med. Chem. 1643-1645, 1983.
2. Burke, T.R., Rice, K.C. and Pert, C.B. Synthesis of 17-methyl and 17-cyclopropylmethyl-3,14-dihydroxy-4,5 α -epoxy-6 β -fluoromorphinans (foxy and cyclofoxy) as models of opioid ligands suitable for positron emission transaxial tomography. Heterocycles, in press.

3. Pert, C.B., Danks, J.A., Channing, M.A., Eckelman, W.C., Larson, S.M., Bennett, J.M., Burke, T.R. and Rice, K.C. [¹⁸F]-3-Acetylcyclofoxy: A useful probe for the visualization of opiate receptors in living animals. FEBS Letters, in press.
4. Rothman, R.B., Danks, J.A., Jacobson, A.E., Burke, Jr., T.R., Rice, K.C. and Pert, C.B. Tritiated-6-beta-fluoro-6-desocy-oxymorphone: a highly selective ligand for the opiate mu receptor whose binding is characterized by low nonspecific binding. Neuropeptides, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02183-02 NS

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Is Schizophrenia an Autoimmune Neuropeptide Receptor Disease

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: C. B. Pert Pharmacologist NS, NIMH

Others: R. J. Weber Senior Staff Fellow NS, NIMH

J. G. Knight Guest Researcher NS, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Section on Brain Biochemistry

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The notion that schizophrenia has an important autoimmune component has been around for several decades, but has not previously been subjected to analysis by the most sensitive, modern techniques. We have developed a simple sensitive assay for detecting antibodies directed against human brain found in sera of schizophrenic patients and controls. We are now exploring the frequency of these antibodies in schizophrenics vs. controls and characterizing the molecular properties of the brain antigens involved and their distribution by visualization in rodent and human brain.

Project Description:Objectives:

1. To develop a simple assay for demonstrating brain-directed autoantibodies in schizophrenic sera.
2. To demonstrate the molecular properties of these brain antigens and their distribution in brain tissue.
3. To explore the possibility that the antigens are cell surface neuropeptide receptors which mediate the biochemistry of emotion.

Methods Employed:

A novel filtration and centrifugation assay for detecting brain antigens in sera and the (McLean, et al., Brain Res. 278: 255-257, 1983) method for visualizing antibody distribution patterns in brain.

Major Findings:

1. In collaboration with Dr. Weber, over twenty experiments were performed for the purpose of optimizing the conditions of the antibody detection assay. In an early blind experiment, six of the Clinical Center 4-East ward acute schizophrenics' and controls' sera were examined. The two highest numbers in the assay belonged to the two sickest patients. We utilized the sera from these two patients vs. two controls in every experiment as we worked on optimization. The assay appears to sensitively and repeatedly demonstrate differences between controls and sera from other patients which were recently screened by Dr. DeLisi. The new patient's serum level appeared elevated even after repeated blood sample withdrawals over a period of one year. We know history of this area and are proceeding cautiously.
2. A significant (14%) percentage of hospitalized chronic patients have antibrain antibodies titers outside the control range.

Significance to Biomedical Research and Program of the Institute:

Schizophrenia is a crippling psychiatric disease which affects one percent of the general population. A complete, convincing understanding of its etiology would almost certainly lead to better therapeutic strategies and would place this psychiatric illness in a more "normal" context with other diseases of the body.

Proposed Course:

We must now collect a large number of determinations on many sera to describe the incidence in normal and schizophrenic sera as well as correlating psychotic symptoms with antibody titers over time within one patient. We plan to perform appropriate controls for neuroleptic drug treatment. We plan future experiments to test whether the incidence of antibodies directed against brain are much higher in schizophrenics--and perhaps certain subtypes--than in normal controls,

and that this incidence is not due to neuroleptic drug treatment. If schizophrenia is indeed a virally-triggered autoimmune disease, we will prove it and provide replicatable methods for others.

Publications:

1. DeLisi, L.E., Weber, R.J. and Pert, C.B. Are there antibodies against brain in sera from schizophrenic patients Review and prospectus. Biological Psychiatry, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02185-02 NS

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

What is the Etiology of Small Cell Carcinoma of the Lung

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: C. B. Pert Pharmacologist NS, NIMH

Others: M. R. Ruff Immunologist LM, NIDR

COOPERATING UNITS (if any)

Laboratory of Microbiology and Immunology, NIDR.

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Section on Brain Biochemistry

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

0

PROFESSIONAL

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been incorporated into Z01 MH 02189-01 NS.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02189-01 NS

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neuropeptides and Their Receptors are Shared by the Brain and the Immune System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: C. B. Pert Pharmacologist NS, NIMH

Others: R. J. Weber Senior Staff Fellow NS, NIMH

J. A. Danks Guest Researcher NS, NIMH

M. A. Herkenham Psychologist LN, NIMH

M. R. Ruff Immunologist LM, NIDR

S. M. Wahl Immunologist LM, NIDR

L. M. Wahl Immunologist LM, NIDR

COOPERATING UNITS (if any)

Laboratory of Neuropsychology; Laboratory of Microbiology and Immunology, NIDR

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Section on Brain Biochemistry

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

1.5

PROFESSIONAL:

1.0

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

 (a) Human subjects (b) Human tissues (c) Neither (a1) Minors (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

A literature is rather rapidly developing which demonstrates that many of the neuropeptides previously studied for their behavioral effects and neuroanatomical distributions, have demonstrable and specific effects on components of immune system function. Moreover, some immune system cells have been shown to contain neuropeptides which they presumably use for intracellular communication in some manner analogous to brain. Since neuropeptides as well as their receptors are found distributed in parts of the brain which mediate emotion, it seems exciting, and plausible to suggest that psychosomatic interactions may be mediated by this network.

Project Description:Objectives:

To test the hypothesis expressed in the summary.

To convincingly document receptor mediated effects of neuropeptides in the immune system.

To further document the distribution of neuropeptides and their receptors in brain.

Methods Employed:

A new immunohistochemical assay for visualizing patterns of antibody binding to neuroanatomically preserved sections of rat brain.

A novel and simple radiochemical membrane binding assay for measuring cell surface antigens with monoclonal antibodies.

The Herkenham and Pert method for autoradiographic visualization of brain receptors.

Major Findings:

1. We previously showed that oat cell carcinoma of the lung is characterized by aberrant fetal-like cells which secrete the brain neuropeptide bombesin. We now show that these cells are indeed part of the immune system since they have four surface antigens previously shown to be completely specific to macrophages.
2. Human macrophages can chemotax toward morphine and opiate peptides in a specific receptor-mediated mode, since this effect is reversed completely by the active enantiomer (-)-naloxone, but not affected at all by its inactive enantiomer (+)-naloxone.
3. A comprehensive literature search revealed that we could expect to find effects of endorphins on the immune system.
4. Like opiate receptors, substance P receptors map in areas known to mediate emotion and appetite.

Significance to Biomedical Research and Program of the Institute:

In the last annual report to justify my interest in lung cancer to our Mental Health Institute I wrote "While the study of lung cancer at first may not appear to be directly applicable to the mission of the Institute, an interesting side effect of the terminal stages of oat cell disease is severe psychotomimetic symptoms which may be due to the activation of brain peptide receptors with the neurocirculatory products of the lung cancer cell." Now, the research has led to a new realization that the body's own healing mechanism (macrophages drawn from bone marrow to enzymatically repair damaged lung tissue) may go awry through

mutation to cause disease. This connection provides a provocative "mind-body" link since neuropeptides appear to regulate mood.

If we actually have an experimental handle on the biochemical basis of psychosomatic disease, the significance to biomedical research is obvious.

Proposed Course:

We plan to continue to explore effects of other neuropeptides and their psychoactive drug analogs on the precise and elegant chemotactic system. We will continue to apply the newest methods for mapping the neuroanatomical distribution of neuropeptides and their receptors.

Publications:

1. Ruff, M.R. and Pert, C.B. Small cell carcinoma of the lung: macrophage specific antigens suggest hemopoietic stem cell origin. Science 225: 1034-1036, 1984.
2. Weber, R.J. and Pert, C.B. Opiatergic modulation of the immune system. Central and Peripheral Endorphins: Basic and Clinical Aspects, Muller, E.E. and Genazzani, A.R. (eds.), Raven Press, New York, pp. 35-42, 1984.
3. Ruff, M.R., Wahl, S.M., Mergenhagen, S. and Pert, C.B. Opiate receptor-mediated chemotaxis of human monocytes. Neuropeptides (Europe), in press.
4. Rothman, R.B., Herkenham, M., Pert, C.B., Liang, T. and Cascieri, M.A. Visualization of rat brain receptors for the neuropeptide, substance P. Brain Res., in press.
5. Rothman, R.B., Danks, J.A., Herkenham, M., Cascieri, M.A., Liang, and Pert, C.B. Autoradiographic localization of a novel peptide binding site in rat brain using the substance P analog, eledoisin. Neuropeptides, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

701 MH 02190-01 NS

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Distribution and Properties of Opiate and Other Brain Receptors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	C. B. Pert	Pharmacologist	NS, NIMH
Others:	R. B. Rothman	Guest Researcher	NS, NIMH
	J. M. Hill	Guest Researcher	NS, NIMH
	S. McLean	Staff Fellow	NS, NIMH
	M. A. Herkenham	Psychologist	LN, NIMH

COOPERATING UNITS (if any)

Laboratory of Neuropsychology, NIMH

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Section on Brain Biochemistry

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

1.3

PROFESSIONAL:

1.0

OTHER:

0.3

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects
 (b) Human tissues
 (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We continue to study the distribution of brain neurotransmitter or drug receptors with our newly developed autoradiographic method. We report new findings on a more parsimonious explanation of the confusing escalation of multiple opiate receptor subtypes. Studies continue to support a type I, conformationally malleable, evolutionarily recent receptor that can assume many conformational states and a type II receptor rigidly locked into a delta conformation. Applications of strategies useful for studying opiate receptors have revealed, for example, that alpha-bungerotoxin receptors and cholinergic nicotinic receptors in rat brain have two uniquely separate brain distributions.

Project Findings:Objectives:

1. To map the neuroanatomical distribution of various chemically coded pathway in brain.
2. To understand the neuroscientific significance of "multiple" receptors.

Methods Employed:

1. Newly developed in vitro autoradiography - unfixed frozen brain tissue is melted onto slides, incubated in appropriate radioactive ligand to label receptors, washed serially, dried rapidly, fixed with paraformaldehyde vapors and dipped in radiosensitive liquid emulsion for autoradiographic visualization.
2. Sophisticated computer analysis of receptor binding kinetics is used to rigorously define conditions of multiple opiate receptor binding.
3. For the first time we bring together rigorous kinetic analysis with autoradiographic distribution of binding sites.

Major Findings:

1. One opiate delta receptor appears conformationally fixed, while the other appears capable of assuming mu, delta and kappa conformations.
2. We mapped for the first time the brain's nicotinic cholinergic receptors and find them in similar limbic parts of brain with neuropeptide receptors.
3. We demonstrate that alpha-bungarotoxin sometimes thought to label nicotinic receptors clearly labels distinctly different population of receptors since patterns at many levels of rat brain in adjacent sections are strikingly different for the two ligands.

Significance to Biomedical Research and Program of the Institute:

Pinpointing neurochemically coded tracts will enable us to determine the functional significance of each newly discovered pathway. The method can be used on human brain and ultimately should give information about the contribution of various neurochemically coded tracts to pathology.

Proposed Course:

We plan a sophisticated biochemical and immunological approach to further defining the molecular nature of opiate receptors. The type I opiate receptor complex with its advanced evolutionary accumulation in the forebrain of humans seems particularly worthy of further study (see "Toward the Visualization of Opiate Receptors in Living Human"). We plan to study the brain distribution of insulin, transferrin, and their receptors to further demonstrate the breakdown in the distinction between "neuropeptides" and hormones.

Publications:

1. Clarke, P.B.S., Pert, C.B. and Pert, A. Autoradiographic distribution of nicotine receptors in rat brain. Brain Res., in press.
2. Clarke, P.B.S., Schwartz, R., Paul, S.M., Pert, C.B. and Pert, A. Comparison of rat brain nicotine receptors labeled with ^3H -acetylcholine, ^3H -nicotine or ^{125}I -alpha-bungarotoxin. J. Neuro. Sci., in press.
3. Rothman, R.B., Schumacher, U.K. and Pert, C.B. Binding of radiolabeled opiates to slide-mounted sections of molded minced rat brain: a novel method for conducting radioreceptor assays. Neuropeptides 3, 493-499, 1983.
4. Rothman, R.B. Bowen, W.D., Schumacher, U.K. and Pert, C.B. Effect of beta-FNA on opiate receptor binding: preliminary evidence for two types of mu receptors. Eur. J. Pharmacol. 95: 147-148, 1983.
5. Rothman, R.B., Bowen, W.D., Bykov, V., Schumacher, U.K. and Pert, C.B. Preparation of rat brain membranes greatly enriched with either type-I-delta or type-II-delta opiate binding sites using site directed alkylating agents: evidence for a two-site allosteric model. Neuropeptides 4: 201-215, 1984.
6. Rothman, R.B., Danks, J.A. and Pert, C.B. Ionic conditions differentially affect ^3H -DADL binding to type-I and type-II opiate delta receptors in vitro. Neuropeptides 4: 261-268, 1984.
7. Rothman, R.B., Pert, C.B., Jacobson, A.E., Burke, Jr., T.R. and Rice, K.C. Morphine noncompetitively inhibits [^3H]leucine enkephalin binding to membranes lacking type-II delta binding sites: evidence for a two-site allosteric model. Neuropeptides 4: 257-260, 1984.
8. Rothman, R.B., Bowen, W.D. and Pert, C.B. Autoradiographic localization of the opiate receptor complex in rat brain: relationship to type-I and type-II opiate receptors. Brain Res., in press.
9. Rothman, R.B., Schumacher, U.K. and Pert, C.B. Effect of beta-FNA on opiate delta receptor binding. J. of Neurochem., in press.
10. McLean, S., Skirboll, L.R. and Pert, C.B. Comparison of substance P and enkephalin in rat brain: an overview using radioimmunocytochemistry. Journal of Neuroscience, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00787-05 LBEB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Brain Mechanisms of Isolation Call in Squirrel Monkey (*Saimiri sciureus*)

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: P. D. MacLean, M.D. Chief LBEB, NIMH

Others: J. D. Newman Research Physiologist LCE, NICHD

COOPERATING UNITS (if any)

Laboratory of Comparative Ethology, NICHD

LAB/BRANCH

Laboratory of Brain Evolution and Behavior

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

TOTAL MAN-YEARS:

1.3

PROFESSIONAL:

0.3

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Audiovocal communication for maintaining maternal-offspring contact counts as one of three key developments in the evolutionary transition from reptiles to mammals. The isolation (separation) call putatively ranks as the most primitive and basic mammalian vocalization, serving originally to maintain maternal-offspring contact. Previous work on this project utilizing the squirrel monkeys (*Saimiri sciureus*) has indicated that the isolation call is represented in an as yet undefined band of medial frontal limbic cortex and contiguous neocortex. In the continuation of this study an effort is being made to identify thalamic nuclei and related frontal cortical areas involved in the production of spontaneous isolation calls. Ablation of the frontal lobes rostral to the genu of the corpus callosum, but not an ablation forward of the cingulate sulcus, results in an enduring failure to emit spontaneous calls. Aspiration of the frontal limbic cortex of the anterior cingulate, pregenual, and preseptal areas, along with part of "area 32," appears to be sufficient to eliminate spontaneous calls. Ablation of the face area of the supplementary area results in a transitory failure to produce calls. The anterior medial thalamic (AM) and parts of the medial dorsal nucleus (MD) have overlapping projections to parts of the frontal limbic areas. Current findings indicate that destruction of AM is not sufficient to eliminate spontaneous calls, and that additional loss of overlapping projections from MD may be necessary to obtain the deficit.

Others Engaged in Project: R. E. Gelhard
C. R. Harbaugh

Project Description:

Objectives: Three cardinal developments in the evolutionary transition from reptiles to mammals were (1) nursing, in conjunction with maternal care; (2) audiovocal communication for maintaining maternal-offspring contact; (3) and play. Adding to the original findings of Stamm and Slotnick regarding maternal behavior, work in our Laboratory indicates that the behavioral triad in question is represented in the thalamocingulate division of the limbic system. Significantly, there appears to be no definite counterpart of the thalamocingulate division in the brains of reptiles.

The experiments to be described are part of a continuing study focusing on the isolation (separation) call which putatively is the oldest, and most basic mammalian vocalization, serving originally to maintain maternal-offspring contact. In addition to further experimentation on brainstem mechanisms of the call, we are attempting to delimit areas of the medial frontal limbic cortex and neocortex involved in the spontaneous production of the isolation call in squirrel monkeys.

Methods Employed: For obtaining neurobehavioral information relevant to primates we use mature male and female squirrel monkeys of either the gothic- or roman-types described in an accompanying report (Z01 MH 00851-20 LBEB). These two types of monkeys with defined karyotypic differences can be just as readily distinguished by their isolation calls as by their display behavior. For pre- and postoperative testing monkeys are transferred from the animal room to a sound-attenuating chamber. Criterion performance is the production of 20 spontaneous isolation calls within 15 minutes. If a monkey fails to reach criterion, it is tested for its ability to produce responsive calls while listening an additional 15 minutes to conspecific calls. A standard Uher microphone and tape recorder are used to record vocalizations, and spectrograms are generated by a VII model 700 sound spectrograph. Once a monkey reaches criterion performance in two or more tests, it is subjected to surgical ablation of various parts of the frontal lobe or to electrocoagulations of targeted areas of the brainstem.

In an attempt to identify thalamofrontal systems involved in the isolation call, we are testing the effects of both massive and restricted frontal ablations, as well as the effects of bilateral coagulation of nuclear groups projecting to medial frontal limbic and neocortical areas. In connection with Project No. Z01 MH 00851-20 LBEB, we are also obtaining further information about diencephalic and midbrain structures that may be involved in the isolation call.

Major Findings: This year's report includes findings on eight monkeys.

Frontal ablations. In two monkeys an attempt was made to eliminate all frontal lobe structures rostral to frontal plane AP 19 of the stereotaxic brain atlas. This plane is just forward of the knee of the corpus callosum and the

lateral ventricles. In one roman-type male (No. 459-E) the operation involved a bifrontal lobectomy, while in the other animal, a gothic-type male (X-5), it was accomplished by lobotomy. In each case such ablations resulted in a failure of the animals to produce spontaneous isolation calls during the three months of testing before sacrifice. The monkey with a frontal lobectomy proved capable of producing a few calls upon hearing the calls of a conspecific, but the lobotomized animal made no responsive calls. The medial dorsal nucleus in these animals showed extensive degeneration in the dorsolateral quadrants.

In a third animal, a gothic-type male (P-086), the tips of the frontal lobes were ablated rostral to the cingulate sulcus and along frontal plane AP 23. Except for erratic performance occurring after the 12th week, this monkey has continued to achieve criterion in producing spontaneous isolation calls.

In monkey R-5, reported last year, ablation of the rostral limbic cortex, together with medial frontal neocortex, resulted in a permanent failure to produce spontaneous isolation calls. Accordingly, an attempt is being made to delimit the effective areas. In pilot operations an approach was devised to avoid damage to the medial frontal neocortex. A gothic-type male (Z-5) with aspiration of the rostral band of limbic cortex (including the cingulate cortex rostral to AP 14; the pregenual area and adjacent area 32; the subcallosal gyrus and adjoining part of the gyrus rectus) has failed since surgery four weeks ago to produce spontaneous calls. A second gothic-type male (P-085) with an intended coagulation of the subcallosal gyrus and adjoining part of the gyrus rectus has, with one exception, continued to achieve criterion during a three-month postoperative period. Finally, a female gothic-type monkey (C-1) with an anatomically confirmed aspiration of the rostral supplementary area (face representation) did not produce isolation calls for the first three weeks, but achieved criterion by nine weeks.

Thalamic lesions. The anterior medial nucleus and parts of the medial dorsal nucleus provide overlapping projections to the anterior, pregenual, and subcallosal parts of the cingulate cortex, while medial MD projects to the posterior part of gyrus rectus. In one monkey (X-5) a remarkably symmetrical electrocoagulation destroyed the greater part of the anterior medial nuclei, as well as the rostral part of N. reuniens and adjacent central nuclei. There was no alteration in the production of the isolation call during four months prior to the time this monkey submitted to a frontal lobotomy (see above). A second gothic-type male monkey (A-6) with an intended large electrocoagulation of the medial part of the medial dorsal nucleus has produced responsive, but no spontaneous, isolation calls since surgery ten days ago. (Stimulation of part of the medial dorsal nucleus in question is known to produce peeping vocalization, as well as penile erection.)

Additional findings. One monkey (W-5, reported last year) with a dyskinesia and cerebellar ataxia, continued following surgery to produce isolation calls at the preoperative rate. This monkey has proved to have had a symmetrical lesion involving the ventral part of the brachium conjunctivum and the medial part of the substantia nigra. Two other monkeys, not formally included in the present study because of inconsistent preoperative performance, contribute

some findings of interest. One gothic-type male monkey (U-5) which was shown preoperatively to be capable of producing both spontaneous and responsive calls never again made such vocalizations following a bilaterally symmetrical lesion destroying (1) the ansa lenticularis just before its fusion with the thalamic fasciculus; (2) the mammillothalamic and mammillotegmental tracts; and (3) rostral components of the medial longitudinal fasciculus. A second monkey (V-5), with a lesion of the dorsomedial hypothalamic area extending from the level of the fornix to the roof of the third ventricle, proved capable of producing responsive calls. The dorsomedial hypothalamic area is known to receive a strong projection from the periaqueductal gray matter and is a locus where electrical stimulation elicits a variety of vocalizations.

Neuropharmacological facts. As described in a previous report (Z01 MH 00781-03 LBEB), the administration of morphine eliminates the production of isolation calls, whereas its antagonist naloxone reinstates the calls. Curiously, there are no reports of neuropharmacological agents that either induce or enhance various forms of vocalization. Three agents were mentioned in last year's report that had no effect on inducing or enhancing isolation calls. This year five more drugs and the respective doses that failed to affect vocalization in the following squirrel monkeys are: diazepam (0.5 mg) administered to monkeys 7281 and U1; beta-carboline (1.25 mg/kg) to monkey 395K; clonidine (0.1 mg/kg) to monkeys P084 and U1; yohimbine (1.0 mg/kg) to Naamah and U1; and metergoline (1.0-1.2 mg) to Naamah and P345.

Significance to Biomedical Research and the Program of the Institute: As noted, the isolation (separation) call possibly represents the most primitive and basic mammalian vocalization, serving originally to maintain maternal-offspring contact. It is of unusual interest that this vocalization finds representation in the cingulate gyrus which is also implicated in parental care and play, two other forms of behavior that characterize the evolutionary transition from reptiles to mammals. Significantly, in the reptilian brain there appears to be no counterpart of the thalamocingulate subdivision of the limbic system.

A major effort this year has been to learn whether or not, in addition to the frontal limbic cortex, the frontal neocortex is essential for the production of spontaneous isolation calls. The work has revealed that (1) a frontal lobe ablation rostral to the knee of the corpus callosum, but not an ablation forward of the cingulate sulcus, results in an enduring elimination of spontaneous isolation calls; (2) aspiration of the paragenual and preseptal limbic cortex, together with the adjoining part of area 32, appears to be sufficient for preventing the production of such calls; and (3) ablation of the supplementary face area results in only a transitory elimination of the calls.

The anterior medial thalamic nucleus (AM) and parts of the medial dorsal nucleus (MD) are known to have overlapping projections to parts of the frontal limbic areas. This year's findings indicate that destruction of AM is not sufficient to eliminate spontaneous calls, and that the additional loss of overlapping projections from MD may be requisite for obtaining the deficit.

Because of the fatal consequences of any prolonged separation, Nature appears to have assured that maternal-offspring separation in mammals induces severe distress comparable to pain. In recent years claims have been made that for some individuals the traumatic effects of prolonged infant separation from the mother or mother substitute sets the stage for later schizophrenic forms of illness, alcoholism, or drug abuse. Apropos of the present project it is of special interest that the cingulate cortex is rich in opiate receptors and that it has been observed in animal studies that morphine not only eliminates the separation call, but also interferes with maternal behavior.

Proposed Course: To be continued.

Publications:

Invited editorial: MacLean, P.D.: Evolutionary psychiatry and the triune brain. Psychological Medicine 14: 1984 (in press).

MacLean, P.D.: Brain evolution relating to family, play, and the separation call. Arch. Gen. Psychiatry (in press).

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00793-03 LBEB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Brain Iron and Neuroendocrine Regulation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J. M. Hill Visiting Fellow LBEB, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Brain Evolution and Behavior

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

TOTAL MAN-YEARS:

.80

PROFESSIONAL:

.55

OTHER:

.25

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Previous studies in this laboratory have indicated that there is considerable overlap in the pattern of distribution of iron in the brain and the distribution of areas receiving gamma-aminobutyric acid (GABA) nerve terminals. The purpose of the present study is to determine if a disruption of GABA metabolism, by preventing GABA degradation with gamma-vinyl GABA (GVG), affects the accumulation of iron in GABA terminal areas. GVG injected unilaterally into the globus pallidus and adjacent caudate putamen resulted in a significant reduction of iron in the sites of GABA terminals such as the ventral pallidum, globus pallidus, and substantia nigra.

The results of this study provide evidence that the presence of iron in the particular areas in question is related to the metabolism of GABA and accordingly have important implications regarding the effects of iron deficiency on the brain and behavior.

Others Engaged in Project: C. R. Harbaugh

Project Description:

Objectives: Our previous studies on the localization of iron in the brain demonstrated a considerable overlap in the distribution of iron in the brain and areas receiving gamma-aminobutyric acid (GABA) terminals. The present study addresses the following question: Does an alteration of the metabolism of GABA, by the inhibition GABA degradation, affect the accumulation of iron in GABAergic projection sites? Forebrain structures, including the nucleus accumbens, caudate, putamen, and globus pallidus, are the source of GABAergic fibers terminating in the pallidum and substantia nigra. In the present experiment, the globus pallidus and adjacent caudate-putamen or the substantia nigra are injected with a GABA-transaminase inhibitor, and the intensity of iron staining measured in GABAergic target sites.

Methods Employed: In 13 rats Gamma-vinyl GABA (GVG), an irreversible inhibitor of GABA-transaminase, was injected either into the right globus pallidus at its junction with the striatum, or into the right substantia nigra. Control animals were injected with the same amount of saline into the same sites or were subjected to a large electrocoagulation of the globus pallidus and adjacent caudate-putamen. After 48 hours all animals were sacrificed. One of the brains with a forebrain injected with GVG, was processed with GABA-transaminase histochemistry for estimating the size of the injection site. The remaining were perfused with formol saline and processed for iron histochemistry according to the method described previously in Progress Report Z01 MH 00891-07 LBEB. The intensity of the stain in the ventral pallidum, globus pallidus, entopeduncular nucleus, substantia nigra (pars reticulata), and the superior colliculus were measured with a Zeiss microscope equipped with a densitometer.

Major Findings: Compared with the control side, the unilateral pallido-caudato-putamen injections of GVG resulted in a statistically significant decrease of iron in the ventral pallidum, globus pallidus, and substantia nigra, with greatest depletion in the substantia nigra (Fig. 1).

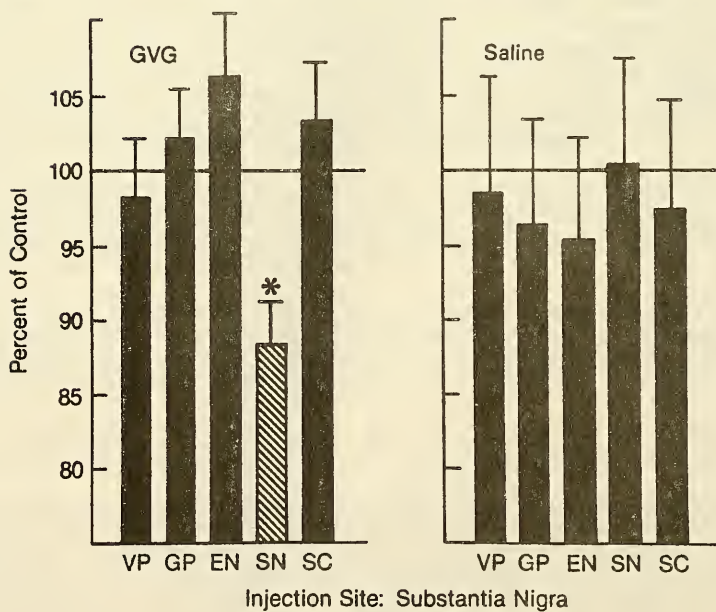
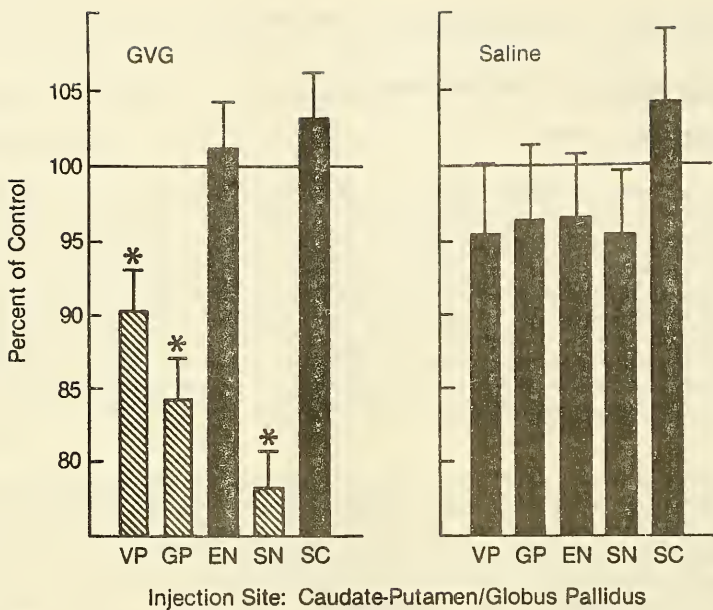
The unilateral injection of GVG into the substantia nigra significantly reduced the amount of iron in the structure itself, but had no detectable effect in the other areas examined. The reduction of iron in the affected areas appeared to occur primarily in the neuropil.

Electrocoagulative lesions of about the same size as the GVG injection site and resulted in no apparent decline of iron in the substantia nigra.

Significance to Biomedical Research and the Program of the Institute: Iron deficiency is, worldwide, the most common nutritional disorder. Iron is found in unusually high concentration in the evolutionary ancient striatal structures of the forebrain. The present study has indicated that the presence of iron is related to the metabolism of GABA. The significance of this finding in regard to mental functioning and behavior is self-evident in the light of the role of GABA as a major inhibiting neurotransmitter in the CNS.

Proposed Course: To be continued.

Publications: None.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00794-01 LBEB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Transferrin Receptors in Rat Brain

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J. M. Hill Visiting Fellow LBEB, NIMH

Others: C. N. Pert Section Chief NSB, NIMH
M. R. Ruff Staff Fellow NIDR, NIH

COOPERATING UNITS (if any)

Section on Brain Biochemistry, Clinical Neuroscience Branch, NIMH; Section on Cellular Immunology, Laboratory of Microbiology and Cellular Immunology, NIDR, NIH

LAB/BRANCH

Laboratory of Brain Evolution and Behavior

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

TOTAL MAN-YEARS:

.45

PROFESSIONAL:

.30

OTHER:

.15

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Autoradiography has been used to localize transferrin receptors in the rat brain and to compare their distribution with that of iron. Up to 85% specific binding of labeled transferrin has been detected on brain sections, and a distinct pattern of receptor distribution, differing from that of iron, has been found.

Project Description:

Objectives: Iron has a specific pattern of distribution in the brain and functions not only in oxidative metabolism, but also in the synthesis, degradation, and binding of neurotransmitters. Transferrin, an iron-binding serum protein, delivers iron to cells by binding to specific receptors on cell surfaces. In addition to iron transport, transferrin may play a role in the growth and development of the brain. The purpose of the present study is to localize transferrin receptors in the rat brain and to compare the pattern of distribution of transferrin receptors with that of iron.

Methods Employed: Slide mounted sections of rat brain were preincubated 5 min in citric phosphate buffer (pH 4.8) and rinsed 15 min in 0.1 M PBS (pH 7.4) containing protease inhibitors. The sections were incubated for 3 hr at 37 C in the pH 7.4 buffer either with ^{125}I transferrin or with ^{125}I transferrin plus excess unlabeled transferrin (10^{-6}M). After incubation the sections were used to generate autoradiograms and to measure specifically bound transferrin by a scintillation counter.

Major Findings: We have found up to 85% specific binding of labeled transferrin on brain sections and a distinct pattern of receptor distribution. The greatest number of transferrin receptors is found in the medial habenula. Moderately high levels are found in the lateral septum, dentate gyrus of the hippocampus, supraoptic and paraventricular nuclei, the interpeduncular and red nuclei, the pontine gray, and locus coeruleus. A group of structures with intermediate density of receptors includes the cerebral cortex, hippocampus, nucleus accumbens, caudate-putamen, bed nucleus of the stria terminalis, substantia nigra (pars compacta), and central gray. The pattern of transferrin receptors differs from that of iron; brain iron occurs in highest concentrations in the ventral pallidum, globus pallidus, substantia nigra (pars reticulata), and circumventricular organs.

Significance to Biomedical Research and the Program of the Institute: Based on the localization of transferrin receptors, the present study has revealed that the areas of high-iron uptake do not coincide with brain areas in which iron accumulates. However, since several sites rich in transferrin receptors project to high-iron areas of the brain, perhaps axonal flow is involved in the transport of iron to the areas in which it accumulates. For example, the iron-rich interpeduncular nucleus receives its major input from the medial habenula, the brain area with the highest density of transferrin receptors, while the nucleus accumbens, caudate, and putamen have numerous transferrin receptors, and their efferents terminate in the iron-rich pallidum and substantia nigra.

It is evident from this study that when considering the effects of iron deficiency, one needs to take into account the areas with high transferrin receptors, as well as high iron.

Proposed Course: To be continued.

Publications: None

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00820-01 LBEB

PERIOD COVERED

October 1, 1983, to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Behaviors of Rats Associated with their Vocalizations.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: John B. Calhoun Chief URBS, LBEB, DIRP, NIMH

Other: James L. Hill Visiting Associate URBS, LBEB, DIRP, NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Brain Evolution and Behavior

SECTION

Unit for Research on Behavioral Systems

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

TOTAL MAN-YEARS:

0.9

PROFESSIONAL:

0.4

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Analog to digital conversions of vocalizations of rats, the F2 of a 6-way cross of wild and domestic strains of rats (Rattus norvegicus), were precisely associated with behavior related to the vocalization. The vocalization data base consists of thirty 45-minute samples for each of two populations of 40 rats each. Dictated observations, concurrent with recording of vocalizations, provided details of individual and inter-individual behavior. On the average there was one such detailed statement about behavior during each 15 seconds of vocalization recording. The present analysis of these data is providing insights into (a) crowding induced changes in vocalizations in contrast to (b) changes in vocalizations characterizing rats which have learned cooperative behaviors.

Project Description:

Objectives: To develop a classification of associated vocalizations and behaviors of rats. Such a taxonomy based on numerous examples of each type of vocalization and behavior set, will enable us to gain a better understanding of the role of vocal communication in rat societies.

Methods Employed: The technical details of recording and analyzing rat vocalization were reported in Z01 MH 00837-01 LBEB to -05 LBEB. The subject populations of rats (*Rattus norvegicus*), on which recordings of vocalization were made, have been described in Z01 MH 00836 LBEB, Z01 MH 00848 LBEB, and Z01 MH 00850 LBEB. Thirty 45-minute samples of vocalizations were recorded for each of two populations of rats. Dictated records of behavior were simultaneously made. On the average one set of behaviors was recorded each minute. These unstructured observations were then transformed into computer compatible, grammatical statements as to the nature, time and place of interactions of specific animals. On the average there were about 180 such structured statements for each 45-minute period of recording.

Major Findings: Coordination of four different timing components in our data recording system provided precise association between vocalizations and behavior. Samples are shown in the accompanying Figure 1. Samples, such as this, of association between behavior and vocalization, represent just the first step of analysis. Several programs to produce statistical analyses or graphic displays have been developed to enable the preparation of a comprehensive characterization of the changes in the pattern of vocalizations that occur across the time of expression of the associated set of behaviors.

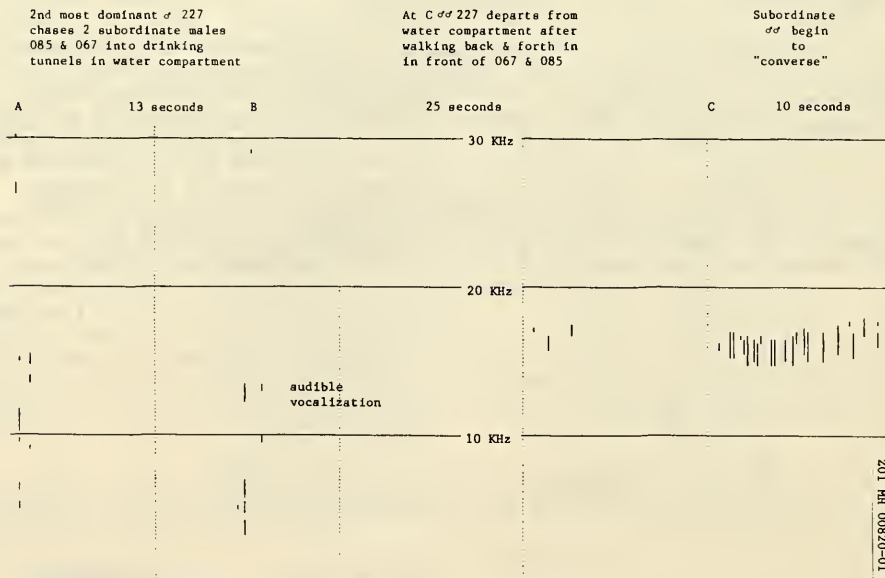


Fig. 1: 48 Second Vocalization Sequence, Universe 34, URBS DAY 4778, Period 6.

Significance to Biomedical Research and the Program of the Institute: This study of vocalization is a special aspect of the larger study (Z01 MH 00836 LBEB and Z01 MH 00848 LBEB) of the influence of acquisition of cooperative behavior on reduction of stress accompanying crowding. We anticipate that final results of the present project can serve as an animal model of (a) changes in vocal communication induced by stress related to crowding, and (b) the alterations in vocalizations associated with acquisition of cooperative behaviors as the population more than doubles. This latter aspect is particularly important because it is pertinent to understanding the forces and changes associated with the initiation of the modern phase of human cultural evolution some 40 millennia ago.

Future Course: During the next year we anticipate assembling 300 or more documented examples of the types of vocalizations associated with particular behaviors. An adequate classification of the relationships between vocalizations and behavior should result from a sample of this size.

Publications: None

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00847-04 LBEB

PERIOD COVERED

October 1, 1983, to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Role of the Neocortex in Coping with Complexity

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: James L. Hill Visiting Associate URBS, LBEB, DIRP, NIMH

Other: John B. Calhoun Chief URBS, LBEB, DIRP, NIMH
Paul D. MacLean Chief LBEB, DIRP, NIMH

COOPERATING UNITS (if any)

None

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SECTION

Unit for Research on Behavioral Systems

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

TOTAL MAN-YEARS:

1.5

PROFESSIONAL:

0.9

OTHER:

0.6

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Pregnant female rats injected with Methylazoxymethanol-acetate (MAM) on the 15th day of gestation produce offspring with marked micrencephaly. The greatest reduction in brain size is seen in the cerebral hemispheres which are reduced to about 60 per cent of normal size: the structures most affected in the cerebral hemispheres are the posterior neocortex and limbic cortex. The micrencephalic rats survive and reproduce in complex social/physical environments, but exhibit a reduced capacity to perform appropriate social and parental behaviors in two main respects: (1) they become involved in many more wound-resulting aggressive encounters and (2) they desert and cannibalize their young at a much higher rate than do controls. These differences in behavior occur predominantly during the initial phases of exposure to the complex situations, and, because later on the animals are somewhat better able to cope with these situations, it is suggested that the brain-damaged individuals adapt more slowly to new, stressful circumstances. Evidence based on brain weight and histological examination of the brains suggests that, not only the reduction in size, but, also the associated disorganization of the cytoarchitecture of the cerebral cortex are important factors contributing to the abnormal behavior.

Project Description:

Objectives: The objective of this study was to determine if micrencephalic rats with a great reduction of neo- and limbic cortex are able to develop appropriate behaviors for surviving and successfully rearing young over an extended period while living as groups in complex environments. The specific objective during the last year of the study has been to evaluate the effect of the treatment on brain size and structure, and to attempt to relate these effects to behavioral differences.

Methods: The procedures followed for inducing micrencephaly and assessing the behavior of rats in this study have been described in detail in Z01 MH 00847-01 LBEB through 03; only an outline of the procedures need be presented here. Micrencephaly was induced in rats by injecting Methyazoxymethanol-acetate (MAM) into pregnant dams on day 15 of gestation. The behavior of MAM-treated groups of rats was compared with that of groups of controls housed under similar conditions of population density (2 levels: 4 versus 8 pairs of adult rats) and environmental complexity. All ninety-six rats in the eight habitats were surgically implanted with a passive resonator which enabled their activity to be monitored by a computer controlled data acquisition system. Data on reproductive and physical condition of adults, and the numbers, weight and condition of pups, were recorded during weekly surveys of all animals. The location of each adult's nest site, and the estimated age of all pups were recorded daily. Behavioral observations were made during daily inspection, and at intervals from the ceiling viewing window of each habitat. During the latter observations, the occurrence of ultrasonic vocalizations and associated behaviors were recorded. Observational data, the weekly survey data, and daily inspection data were pooled to determine the social/ reproductive structure within each environment. Reproductive performance of the rats in the environments was compared with that of bisexual pairs of their siblings housed in standard laboratory cages. At autopsy, the general condition; body weight; weights of spleen, kidneys, and adrenals; four measures of skull size; and number of placental scars in females were recorded for comparisons.

At the time of sacrifice the brains of all animals were fixed in situ and after a transverse section at the level of the obex were removed for weighing. Of the 220 brains, 10% were embedded in gelatin and serial frozen coronal sections were cut at 20 microns. Facing sections at regular intervals were alternately stained for cells and fiber.

Major Findings: Cerebral changes. The mean weight of MAM-treated brains of both sexes is about 67% of that of control brains (Figure 1). The caudal extent of the cerebral hemispheres is so reduced that the third ventricle and most of the midbrain are visible when the brain is viewed from the dorsal aspect. The exposure of the third ventricle occurs because the corpus callosum is absent except for the rostral portion above the septum.

Frontal cerebral sections of MAM-treated rats are about 60% of the area of corresponding sections of the controls. There is a far greater diminution of the cortex in the posterior half than in the rostral half of the brain. The hippocampus is exposed caudally because of the diminished retrosplenial limbic cortex and neocortex. The septum, amygdala, and corpus striatum are reduced

in size but normal in appearance. The thalamus appears less reduced in size than the cerebral mantle. The midbrain, cerebellum and medulla are not discernably affected.

The dorsolateral frontal neocortex, as well as the frontal limbic cortex and the piriform cortex, appear relatively normal as compared with the marked diminution in thickness and disruption of the layers of parietal and occipitotemporal neocortex and of the limbic cortex of the posterior cingulate, retrosplenial, and entorhinal areas. In some brains groups of fibers appear to separate from the internal capsule, cingulum, and corpus callosum and streak through the cortex in a disorderly pattern. Wherever the aberrant fibers make tangles and whorls the picture recalls that of status marmoratus. A notable and regular finding is the appearance of an ectopic mass of cells resembling a cross section of the tail of the caudate nucleus that is located dorsomedial to the junction of the head of the caudate nucleus and corpus callosum. These masses possibly represent an outgrowth of matrix cells normally seen at the site of the dorsolateral ventricular cleft of this region. Masses of the same type of cells are regularly seen as invasions of areas CA2-CA1 at one or two loci in the dorsal hippocampus.

Behavioral changes. That MAM-treated rats exhibit a higher incidence of aggressive behavior (as indicated by wounds) and exhibit poorer parental behavior (as indicated by the number of pups reared to weaning age) has been discussed previously in Z01 MH 00847-03 LBEB. The relationship between aggression and brain size is most clearly seen among males: when both MAM-treated and control males are taken simultaneously, there is a significant correlation between decreased brain size and a higher number of wounds ($r = -0.46, p = 0.001$). The greatest number of the wounds (84%) were found on those areas of the body known to be most vulnerable to injury in subordinate or low-ranking males. The inverse relationship between brain size and number of wounds was more clearly evident among control ($r = -0.49, p = 0.02$) than among the MAM-treated males ($r = -0.38, p = 0.07$), suggesting, perhaps, that there may be a structural as well as a size component to the relationship.

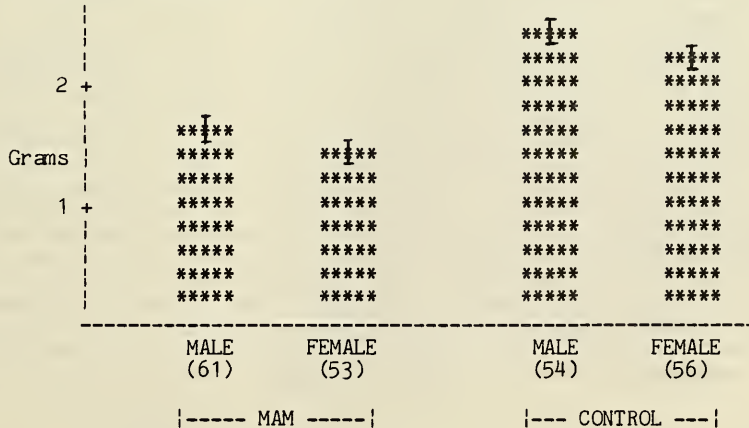


FIGURE 1: Mean brain weights + 1 SD. Sample size ().

There are significant positive correlations between brain size and the number of pups born to ($r = 0.41$, $p = 0.0002$) and weaned by ($r = 0.31$, $p = 0.005$) females when both treatments are considered simultaneously, but not independently since it is only among MAM-treated females that the association between brain size and number of pups weaned approaches significance ($r = -0.27$, $p = 0.09$).

Significance to Biomedical Research and the Program of the Institute.

This long-term study of an animal model system has provided data which show that individuals with micrencephaly marked by severe alteration of the limbic and neocortex in the posterior half of the hemispheres require longer to learn to cope with complex social/physical environments than do normal individuals. Information about relationships between brain damage and reduced capacity to cope with environmental and social complexity not only contributes to the knowledge of brain function, but also provides essential background for the understanding and treatment of mental retardation.

Future Course: After final histological examination of the brains is complete this project will be terminated with submission of a written report.

Publications: none.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00849-02 LBEB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Resting Time Residence in a 7-Generation Population of House Mice

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: John B. Calhoun Chief URBS, LBEB, DIRP, NIMH

Other: James L. Hill Visiting Associate URBS, LBEB, DIRP, NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Brain Evolution and Behavior

SECTION

Unit for Research on Behavioral Systems

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

TOTAL MAN-YEARS:

1.8

PROFESSIONAL:

0.4

OTHER:

1.4

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A population of house mice (*Mus musculus*) developing out of parental C x A F1 hybrids was allowed to increase to 8x optimum density in staged phases, thus permitting the survival each 200-days of sufficient new generation animals to double the size of the population at the beginning of each of seven successive 200-day periods. At about 118 days of age young mice begin to establish membership in adult social groups. An algorithm was developed from data on place of sleeping at time of capture to define episodes of range stability and instability with reference to residence within a 16-cell habitat. Each habitat cell was designed to facilitate optimal relationships among a group of 12 adults. The proportion of the total population characterized by residential stability each successive week was calculated for the socially active adults of 118-565 days of age. Residential stability dropped exponentially from 95% to 61% as crowding increased over the central 150-week history of the population, during which time the population increased to 8x optimum. However, episodes of increased residential stability were evident during each new generational cohort period as members of that cohort attained the beginning of the "prime adult" phase of behavioral development at 172 days of age. Such times of inter-period increase in residential stability will now be used to determine the major members of the group inhabiting each cell during each 200-day period. Findings on groups will then be used to analyze changes in behavior accompanying increased crowding.

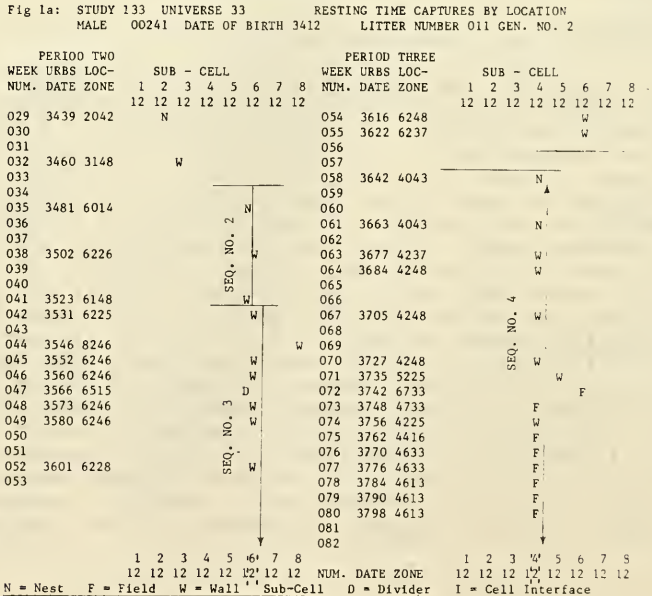
Project Description:

Others Engaged in Project: Garrett A. Bagley, Computer Programmer.

Objectives: To determine the core (sleeping time) residential stability of a population of mice over its 223-week, 7-generation history. A major purpose of the current phase of analysis of this project is to identify the span of maximum residential stability during each of the circa 200-day periods that fall between the dates of establishing successive pairs of generations. This information will allow the determination of group membership in each of the 16 cells of the habitat during each of the 6 major intergenerational periods. Findings on the grand total of 96 groups will provide data for the analysis of the effects of increasing crowding on the behavior and health of members of the population. Identification of these 96 groups completes the first 5 of the 10 steps outlined in Z01 MH 00849-01 LBEB for the analysis of this study, the previous progress of which was described in prior annual reports Z01 MH 00835 LBEB and Z01 MH 00848 LBEB.

Methods: An algorithm was developed from survey capture data to determine whether each mouse in this study was residentially stable or unstable each day of its life. This determination was obtained for the full life span of more than over 3,500 subjects. Graphic displays (Fig. 1) helped develop the

Fig. 1. Resting Time Residence Stability Basic Displays on all 3500 Subjects



algorithm, as well as depict the final result. Then data sets of the proportion of age classes, or the total population, that were stable across the 223-week population history were generated. Group membership for each of the 16-cells of the habitat was then determined for times of maximal residential stability.

Major Findings: Figure 1a displays core residential history for one mouse (No. 0241) over two of the several consecutive circa 200-day periods of its life. Since most mice lived more than 565 days, the residential display of each mouse's history usually encompasses 3 to 5 such circa 200-day periods. Figure 1b summarizes the six stable residence sequences for male 0241. The

Fig 1b. Start and End of Stable Residence Sequences

TAC NO	SX	GN	ST	DOB	NC	FC	LC	SDAY	EDAY	CELL	SC	LDAY	SEQ NO
241	1	2	0	3412	3	1	4	3412	3467	22	3	4067	1
241	1	2	0	3412	3	5	7	3474	3527	61	1	4067	2
241	1	2	0	3412	10	8	17	3528	3628	62	3	4067	3
241	1	2	0	3412	17	18	34	3636	3824	42	1	4067	4
241	1	2	0	3412	5	35	39	3825	3910	41	1	4067	5
241	1	2	0	3412	9	40	48	3911	4067	42	0	4067	6

SX = SEX; 1 = Male; 2 = Female. GN = Generation (1 to 8). DOB = Date of Birth.
SDAY & EDAY = Start & End of a Stable Sequence. SEQ. NO. = SEQUENCE NO.

major phase of active adult life covers the time span of 118 to 565 days of age. Figure 2 shows the change in residential stability for the population across its 223-week history. Optimum population density of adults (100 to 200) characterized periods 1 and 2, during which a 90% or more residential stability was usually maintained. After this, residential stability declined as density

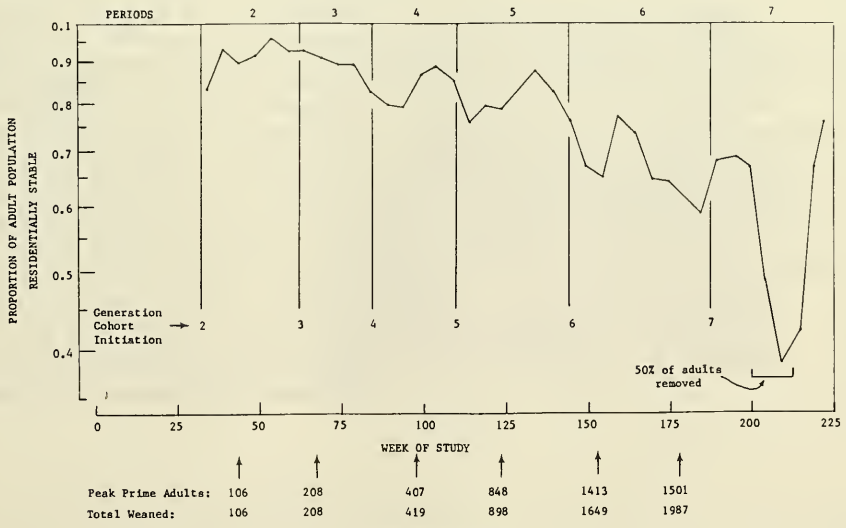


FIG. 2: RESIDENTIAL STABILITY

of the population, and the average age of its members, both increased. However, four episodes of increased residential stability occurred. These closely approximated the time over which the members of the new generational cohort moved into the prime adult age class at about 172 days of age. This identification of these major episodes of residential stability during each of the last six periods of the population history will greatly facilitate identification of the major group members in each cell during each period. Finally, it may be noted that residential stability markedly increased near the end of the study following removal of adults of all generations except the last one; however, an increase in rate of becoming residentially unstable accompanied the social disruption produced during the weeks of removal of the older adults.

Significance to Biomedical Research and the Program of the Institute.

It has long been known that increase of mental illness accompanies change of residence of humans beings. Likewise, it has been learned from our studies that increased crowding in mice results in an increase of behavioral anomalies. The present study with mice presumably may serve as an animal model of how (1) population density, (2) social disorganization, (3) change of residence, and (4) behavioral change affects both individual and social behaviors, as well as capacity to cope.

Future Course: The Johns Hopkins University Press has presented the opportunity for publication of an integrated account of our two large-scale studies of crowding in rodents conducted over the last 10 years.

Publications: none

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00850-02 LBEB

PERIOD COVERED

October 1, 1983, to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cooperation Induced Modification of Behavior in Rats

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: John B. Calhoun Chief URBS, LBEB, DIRP, NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Brain Evolution and Behavior

SECTION

Unit for Research on Behavioral Systems

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

TOTAL MAN-YEARS:

1.5

PROFESSIONAL:

0.3

OTHER:

1.2

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Two populations, each of 40 wild x domestic 6-way crosses of rats (Rattus norvegicus), were studied over an 18-month period in interconnected 33-compartment habitats. Passive resonators implanted under the abdominal skin permitted computer recording of the identity of each rat each time it passed through "portals" connecting adjoining compartments. Time, place, and direction of travel were simultaneously recorded. Males aggregate in the vicinity of compartments between which estrous females move. A "Close Space-Time Association" (CSTA) index was developed to calculate the degree of male-female associations. On days of estrus there is one hour of extremely heightened activity by the female. The CSTA index, during the hour of her heightened activity, is the sum of the number of males which enter a compartment within 5 seconds before, or 5 seconds after, the time that the female entered it. This index is of the order of 45x that of comparable hours on non-estrous days. Calculating this index for hours of high activity each day now makes it possible to plot the course of estrus over the lifetime of all females in the populations. Since group dynamics are markedly modified by estrus it will now be possible to contrast data between estrous and non-estrous days.

Project Description:

Objectives: To identify each day over an 18-month period which of 40 females (each a member of one of two, 20 male + 20 female, social groups) is in estrus. Since group dynamics markedly change from estrous to non-estrous days, it requires analysis of data obtained under both conditions to develop an adequate understanding how changes in the estrous cycles of females in social groups affect: hierarchy; social velocity; strength of social bonding; cooperative behavior; home range; circadian phenomena; parenting; subgroup formation; vocal communication, growth; health; and behavioral adequacy.

Methods: Two populations of rats, (*Rattus norvegicus*), developed from crosses between 2 wild types and 4 domestic breeds, were maintained for 18 months in separate habitats, each consisting of a network of 33 interconnected compartments. Each population contained 20 males and 20 females. A passive resonator coil implanted under the skin of each rat permitted computer recording of its identity, along with the time, location, and direction of travel as it passed through the "portal" that permitted movement between any two adjoining compartments of the habitat. Eight hours of detailed observations were made of each population, each 21 days. A total of 32 females in each of the populations were observed to be in estrus as judged by each engaging in an intense and prolonged episode of mating. These 64 females were screened down to 3 pairs, one member of each of which came from a different population, than the other female of the pair. The members of each pair had to meet a rigorous set of criteria, including: (1) both were nearly the same age; (2) no other female of the subject's population was in estrus on the observation day; (3) the portal passage record includes three days of data before and/or after the day of estrus. This latter permits the female to be used as her own control. These activity records were studied in detail in order to establish criteria to guide development of programs through which the computer could identify each episode of estrus occurring within the two-thirds of the days of the lifetime of each female for which movement data were continuously recorded. Part of the analyses for one (female 152) of these six females is presented below to exemplify the strategy pursued.

Major Findings: Typically there is a single hour (Fig. 1) during the day of estrus that is characterized by a level of activity exceeding that during the rest of that day, as well as on all non-estrous days. Males in close proximity to a female will usually make early contact with her. A single index of Close-Space-Time-Association (CSTA) proved particularly useful in determining whether a female was in estrus. With reference to a female entering compartments, the CSTA is the sum of (1) all occasions when males enter the compartment within 5 seconds before the female enters, or (2) within 5 seconds after she enters. The CSTA count per hour provides this Index. One female, No. 152 of Universe 34, is here shown as a test case, serving as her own control. Mean CSTA data for the 3 pre-estrous days were calculated for the same hour as the estrous hour, plus an adjoining hour of high activity. As seen in the table below, the CSTA Index for this test-case animal on its estrous day is 45 times that for the prior three non-estrous days.

Both visual observation and the CSTA index establish that a particular subset of males is most closely associated with a particular female on her day of estrus. Thus the data for such males, as is the case of the "6 High CSTA"

males in the table below, are particularly useful in distinguishing estrous from non-estrous days.



FIG. 1: Estrous vs. non-estrous activity

CLOSE SPACE-TIME ASSOCIATIONS INDEX (PER HOUR) FOR ♀ 152,
OF UNIVERSE 34 RE ESTRUS ON URBS DAY 4525

Hour(s)	Category of Male Respondents			No. of Portal Passages of ♀ 152
	6 High CSTA Males	7 Low CSTA Males	TOTAL	
Mean of 6 Non-estrous	1	3	4	183 (6 hours)
1 estrous maximum (URBS Day 4525)	177	4	181	159 (1 hour)

Significance to Biomedical Research and the Program of the Institute:

Much firmer insights into behavior and group dynamics may be obtained when it is possible to determine on which days there are one or more females in estrus. Individual behaviors, as well as relations among individuals, drastically differ between estrous and non-estrous days. Our present ability to identify most estrous females from computer recorded data takes us a step closer to understanding, through an animal model, how relations within a complex human social system culminate in either enhancing or disorganizing both individual behavior and intragroup phenomena. Our research strategy may be of more significance than the specific results of our research over the past few years. A synopsis of the origin and implication of this research strategy is here presented because of its implications for the course of research intended to culminate in conclusions helpful in resolving major behaviorally related problems, whether individual or societal.

In 1968 as a part of my A.A.A.S., Moving Frontiers of Science Lecture titled: "Space and the Strategy of Life", I introduced a new theory of human cultural, behavioral, and conceptual evolution. Basically this theory holds that the amount of useful information flowing over the communication network of a population is proportional to the number of individuals in the population, and that the number of different kinds of concepts (ideas, conclusions, constructs, hypotheses, theories, etc.) created within, and circulated through, the population is proportional to the square root of the size of the population. Since increase in the diversity of concepts permitted an ever larger proportion of the population to survive to reproductive age, the world population increased at a greater than exponential rate from about 40 millennia ago to about the present. During this time, within which the human world population increased a thousandfold, the capacity to develop and utilize concepts increased about 33 fold.

This basic formulation should be applicable to any research effort purporting to develop understanding of how behavior is modified within complex societal settings and histories. Our recently completed set of studies, initiated in 1974 and 1975, assumed that all animal model studies up to 1975, that focussed on behavioral change in the context of group and population dynamics, were of such simplicity as to reduce the likelihood of anyone of them generating many important concepts. Therefore, we took great care to increase: (1) the number of variables operating in our studies; and (2) the quantity and accuracy of data collected. These two steps should have the same type of relation to the the generation of new concepts as did the flow of more information between people as the human population increased in numbers. The number of concepts generated by the strategy pursued in our present set of projects should be over 10x the number derived from any prior animal model studies of the dynamics of behavior transpiring in large groups of individuals. If this proves to be a correct assessment, then our strategy of research inquiry gains importance as a model to increase the effectiveness of research pursued by others.

Proposed Course: The Johns Hopkins University Press has presented the opportunity for publication of an integrated account of our two large-scale rodent studies conducted over the past ten years. Our major efforts will be to prepare such a manuscript.

In specific, as a first step in structuring this presentation, we will be conducting a large number of analyses of how the twelve aspects of behavior mentioned above under "Objectives" are influenced by the presence of estrous females. This forms 24 cells of analysis. Since the project is comprised of (1) an experimental population whose members have learned cooperative behavior, and (2) a control population whose members have not, the full cells of analyses are $2 \times 24 = 48$. Over these 48 cells, we will then focus on how the brain influences the duration and sequence of behaviors with respect to the 12 categories of behavior recognizable from their duration and place of occurrence by the computer recorded data on activity.

Publications: none.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00851-20 LBEB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Brain Mechanisms of Display Behavior in Squirrel Monkey (*Saimiri sciureus*)

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: P. D. MacLean, M.D. Chief LBEB, NIMH

Others: J. D. Newman Research Physiologist LCE, NICHD

COOPERATING UNITS (if any)

Laboratory of Comparative Ethology, NICHD

LAB/BRANCH

Laboratory of Brain Evolution and Behavior

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

TOTAL MAN-YEARS:

1.4

PROFESSIONAL:

0.4

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Terrestrial vertebrates utilize four main displays in prosematic (i.e., non-verbal) communication. Since the mirror display of the gothic-type squirrel monkey combines features of three of the displays, it has been used in this long-term project for identifying cerebral structures that integrate the somatic and autonomic components of such displays. In earlier work it was shown that the medial segment of the globus pallidus (and its projecting pathways) is a site of convergence of neural systems involved in the evocation and performance of displays. The present project provides the additional information that (1) destruction of the dorsomedial hypothalamic area between the ansa lenticularis of each side practically eliminates the genital autonomic component of the display; (2) the greater part of the ventromedial nucleus of the hypothalamus may be destroyed without affecting the display; (3) coagulation of the ansa lenticularis near its fusion with the thalamic fasciculus results in a transitory elimination of the display, with recovery except for a deficit in vocalization apparently due to destruction of fibers leading to the medial longitudinal fasciculus; and (4) interference with ascending neocerebellar influences does not affect the regular performance of the display. Finally, evidence has accrued that destruction of medial frontal limbic and neocortical areas known to be implicated in vocalization, results in an alteration and marked reduction of the vocal component of the display.

Others Engaged in Project: R. E. Gelhard
C. R. Harbaugh

Project Description:

Objectives: Terrestrial vertebrates (reptiles, birds, and mammals) utilize four main displays in prosematic ("non-verbal") communication. As in lizards, such displays may be characterized as (1) signature, (2) challenge, (3) courtship, and (4) submissive displays. The purpose of this long-term project is to identify cerebral structures that integrate the somatic and autonomic components of species-typical displays. For such studies relevant to primates, we have used a subspecies of squirrel monkeys that consistently performs a mirror display incorporating features of the signature, challenge, and courtship displays. Prior work on this and related projects has shown that in species as diverse as monkeys and lizards, the striatal complex is basically implicated in the evocation of displays. In the present phase of the work we are attempting a further definition of the role of tegmental pathways in the performance of displays, as well as obtaining information about the effects of destroying adjacent structures. In addition, an associated study on the isolation call (see 787-05 LBEB) has provided the opportunity to obtain supplemental information about the influence of thalamofrontal systems on display behavior.

Methods Employed: Mature, male, gothic-type squirrel monkeys are used for these studies. The gothic-type monkey is characterized by a white ocular patch that forms a peak over the eye like a gothic arch and is thereby distinguished from the roman-type with a round arch. These two general types of squirrel monkeys show karyotypic differences. Only the gothic-type monkey consistently displays to its reflection in a mirror (see M-NP-LI-17, 1964). Living visually isolated from other monkeys, a monkey is tested twice a day in its home cage in which the elevation of a panel reveals its reflection in a one-way mirror. Major and minor components of the display are scored. The combined occurrence of the major components of the displays--full erection, vocalization, and thigh-spreading--constitutes a "trump" display. After achieving criterion performance of a trump display in 80% of thirty trials, a monkey undergoes surgery for electrocoagulation of targeted structures of the brainstem or aspiration of cortical areas. Postoperatively, animals are tested until they achieve plateau performance in a series of at least three sets of thirty trials. Upon completion of the behavioral studies, serial frontal brain sections are prepared for histological study and for reconstruction of the size and extent of the lesions.

Major Findings: Nine monkeys are included in this year's report.

Supplemental tegmental findings. In previous experiments there were no cases with lesions largely restricted to the locus at which the pallidal projections fuse with the thalamic fasciculus. In monkey U-5 a symmetrical electrocoagulation was made at this locus, but there was also destruction of the mammillothalamic tract at the level where the mammillotegmental tract branches from it. During a six-week period of hypothermia (partially self-compensated by exposure to a heat lamp), this monkey failed to display. Shortly

after recovery from the hypothermic condition, it began to display regularly, but in three sets of thirty trials vocalized in less than a third of the tests. Since the deficit in vocalization resembled that of S-5 described in last year's report, it is presumed to be attributable to destruction of fibers leading to the medial longitudinal fasciculus.

In previous work there were no cases with a complete coagulation of the ventromedial nucleus of the hypothalamus. In monkey Y-5 almost the entire nucleus except for the dorsal part on the left was destroyed. From the very first postoperative day this subject continued to achieve a near-perfect score in its display behavior. It is also notable that there was no alteration of its dietary or drinking habits; no gain in weight; and no change in disposition.

In a third case (V-5) the dorsomedial hypothalamic region above the ventromedial nucleus and between the ansa lenticularis was destroyed. (Electrical stimulation within this region is known to be highly effective in inducing a penile erection, as well as vocalization.) Beginning on the second postoperative day this monkey regularly responded to its reflection by performing the somatic components of the display, but there was no sign of genital tumescence until nearly a month later. Thereafter there was regularly occurring tumescence, but only 1+ in magnitude (scale 1-5).

A fourth monkey (W-5) with an electrocoagulation in the ventral tegmental region of the midbrain, developed a Wilson-like athetosis, an occasional resting Parkinson-like tremor, and a severe intention tremor of the upper extremities. As noted in last year's report, this monkey, after a temporary lapse, regained a near-perfect score in its display behavior. The histological examination has revealed a bilateral symmetrical lesion involving primarily the ventral part of the decussation of the superior cerebellar peduncle and the most medial portion of the substantia nigra (maximum diameter of lesion at AP 4.5).

Lesions involving thalamofrontal systems. In conjunction with the project on the isolation call (Z01 MH 00747-05 LBEB), there was the opportunity to confirm and extend previous findings on the effects of lesions involving thalamofrontal systems. In confirmation of previous findings (Z01 MH 00876-01 LBEB), ablation of the frontal tips rostral to the cingulate sulcus (monkey P-086) resulted in no interference with display behavior. In one monkey (X-5) with a frontal lobotomy rostral to the knee of the corpus collosum, there was an enduring "fragmentation" of the display manifest by a statistically significant decline in the occurrence of vocalization with the display. It is also notable that such vocalizations were less forceful than before surgery. In monkey Z-5 in which the rostral band of limbic cortex was aspirated, a weakening of the display vocalization has been noted during the first 30 trials.

In regard to nuclei projecting to the medial frontal limbic cortex, a symmetrical coagulation of the anterior medial nuclei, together with the rostral part of *N. reuniens*, and midline central nuclei, was accomplished in monkey X-5. This animal continued to achieve near perfect performance in three sets of 30 trials. A second animal (A-6) with an intended large coagulation of the medial dorsal nucleus centered at frontal plane AP 7 is now beginning postoperative testing.

Significance to Biomedical Research and the Program of the Institute:

In prosematic ("non-verbal"), social communication, terrestrial vertebrates rely on four main forms of behavior referred to as displays. The importance of the present investigation lies in its attempt to identify cerebral mechanisms accounting for the evocation and integration of the somatic and autonomic components of displays. Previous work on this project demonstrated that the medial segment of the globus pallidus and its projecting pathways play an essential role in the evocation and performance of a species-typical display characteristic of one variety of squirrel monkeys. This year's research has demonstrated a remarkable dissociation of the autonomic and somatic components of the display by showing that electrocoagulation of the dorsomedial hypothalamic area practically eliminates the genital component of the display without affecting the regular performance of the somatic components. In addition, it has been shown (1) that the destruction of the ansa lenticularis near its continuation with the thalamic fasciculus results in a transitory elimination of the display, with recovery except for a deficit of the vocalization; (2) interference with ascending neocerebellar influences does not affect the regular performance of the display; and (3) the greater part of the ventromedial nucleus of the hypothalamus may be destroyed without affecting the display. Finally, evidence has been obtained that destruction of medial frontal limbic and neocortical areas known to be implicated in vocalization, results in an alteration and marked reduction of the vocal component of the display.

Proposed Course: To be continued.

Publications:

MacLean, P.D.: A Triangular Brief on the Evolution of Brain and Law. In Gruter, M. and Bohannon, P. (Eds.): Law, Biology & Culture. The Evolution of Law. J. Soc. Biol. Struct. 5: 369-379, 1982

MacLean, P.D.: Brain roots of the will-to-power. Zygon 18: 359-374, 1983.

MacLean, P.D.: Commentary on disorders of the limbic system. Integrative Psychiatry 2: 102-103, 1984.

MacLean, P.D.: Commentary: A brain theory commensurate with Procrustes' Bed. The Behavioral and Brain Sciences 7: 1984 (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00871-08 LBEB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

A Histological Study on the Location of Brain Iron

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J. M. Hill Visiting Fellow LBEB, NIMH

Others: C. E. Fiori Physical Scientist DRS, NIH
C. R. Swyt Physical Scientist DRS, NIH

COOPERATING UNITS (if any)

Office of the Chief, Biological Engineering and Instrumentation Branch, Division of Research Services, NIH

LAB/BRANCH

Laboratory of Brain Evolution and Behavior

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

TOTAL MAN-YEARS:

.50

PROFESSIONAL:

.30

OTHER:

.2

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The study of the localization of iron within the brain has been expanded to include experiments on the effects of dietary iron deficiency on brain iron and others involving x-ray elemental analysis of brain tissue.

A completed pilot study has established the techniques for inducing dietary iron deficiency, obtaining blood samples, and measuring hemoglobin; the Perls'+DAB histochemical test for iron has been modified for very low levels of iron.

X-ray elemental analysis with electron beam imaging and microspectroscopy at the Biological Engineering and Instrumentation Branch, DRS, permits the quantification of iron within individual brain cells and perhaps also within structures as small as organelles. In addition, other metals in association with the iron deposits can be detected. This method can be used to verify the identification of iron accumulations made with the DAB intensification of the Perls' histochemical method for ferric iron.

Others Engaged in Project: C. R. Harbaugh

Project Description:

Objectives: In our previous studies on brain iron and its relation to the anatomy and function of the striatal complex, we have localized iron at both the light and electron microscopic level. The present investigations represent a continuation of these studies and have two main objectives: Study 1 is designed to determine if dietary induced iron deficiency affects the distribution and/or concentration of brain iron. The purpose of Study 2 is to localize and quantify brain iron by means of electron probe x-ray microanalysis.

Methods Employed:

Study 1. After 48 days of age, rats (12 of each sex in each treatment group) are maintained on an iron deficient (2-5 ppm iron) or iron replete (35-40 ppm iron) diet. Weekly measurements of body weight and hemoglobin are obtained. After hemoglobin drops from the normal levels of 14-16 gm/100 ml to 5-6 gm/100 ml (within 5-6 weeks) in the iron deficient group, the animals are perfused with a fixative. Brain sections are processed for the Perls'+DAB histochemical method for iron, and the intensity of iron stain in several iron-concentrating brain sites is determined with densitometry.

Study 2. X-ray microprobe analysis is performed at both the cellular and subcellular levels. For low-level magnification, saline perfused brain tissue is cut at 20 μ m on the cryostat and mounted on pure carbon discs. After dehydration the tissue is analyzed on the electron beam microprobe. For the elemental analysis at the electron microscope level brain tissue is prepared according to methods for routine electron microscopy, with the deletion, where possible, of metal-containing compounds from solutions. In addition, tissue is prepared following the method used for the histochemical detection of iron for electron microscopy (see preceding report Z01 MH 00871-07 LBEB).

Major Findings:

Study 1. To date, the techniques have been perfected for inducing iron deficiency, obtaining blood samples from the rat, and measuring hemoglobin. The Perls'+DAB histochemical test for iron has been modified for very low levels of iron.

Study 2. Initial observations on the globus pallidus indicate that the microprobe analysis is adequate for measuring iron and copper in the perikarya of individual cells.

Significance to Biomedical Research and the Program of the Institute: Dietary induced iron deficiency results in the reduction of whole brain iron. It is not known (1) which brain areas, if any, are affected by iron deficiency; (2) which iron-containing compartments (glial, neuronal, or both) suffer a reduction in iron; and (3) whether or not iron deficiency results in structural

changes in organelles as has been observed in the liver and other iron-concentrating body organs. These are all questions that the present study should be able to provide information about in a year's time.

Proposed Course: To be continued.

Publications:

Hill, J.M., and Switzer, R.C.: the regional distribution and cellular localization of iron in the rat brain. Neuroscience 11: 595-603, 1984.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00881-28 LCM

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Intermediary Energy Metabolism in Mammalian Brain

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Elaine E. Kaufman Research Chemist LCM, NIMH

Others: Thomas Nelson Staff Fellow LCM, NIMH
Louis Sokoloff Chief, LCM LCM, NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Cerebral Metabolism

SECTION

Developmental Neurochemistry Section

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

3.75

PROFESSIONAL:

2.25

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The project which is covered in this report is a study of 1) the biosynthesis and metabolism of γ -hydroxybutyrate (GHB), a naturally occurring, neuroactive compound and 2) the role which this compound plays in the central nervous system. Several pathways of biosynthesis are currently being investigated. Studies of the enzyme which plays a major role in the degradation of GHB are continuing.

A new emphasis will be placed on studies of the role of GHB in the central nervous system, on the relationship of GHB to the opiates and on the mechanisms by which GHB exerts its profound physiological effects. A screening project to detect patients with defects in GHB metabolism has been started.

Project Description:

This project has continued its studies on the origin, mode of action, and metabolism of GHB, a compound which is a normal constituent of mammalian brain and which may function as a neuromodulator or possibly as a neurotransmitter. Where administered in pharmacological doses, GHB produces a flattening of the EEG, a reversible trance-like state, a profound depression of cerebral glucose utilization, and a marked increase in striatal dopamine levels.

This project is directed at understanding the role of naturally-occurring GHB as well as the metabolic pathways involved in its synthesis and degradation. Since the compound is also used as a drug, other important questions arise concerning its pharmacological actions.

GHB is being used clinically (in Europe) as an anesthetic adjuvant and has been used in Canada in the treatment of narcolepsy. New trials with GHB in the treatment of narcolepsy are being conducted in the United States and appear to confirm the promising results obtained in Canada. We have proposed a collaborative study with a group at Stanford University who have an animal model of narcolepsy. We would assay GHB dehydrogenase levels in a group of control and affected animals.

Recently, a new metabolic disease has been found in which the patients have extremely high levels of GHB in both blood and urine, tissues which ordinarily contain only trace amounts of GHB. These patients are mentally and physically retarded with neurological abnormalities. The discovery of this disease as well as the fact that GHB is being used clinically underline the need to know more about the pathways of biosynthesis and of degradation for GHB as well as the factors which regulate these pathways.

One of the main efforts, therefore, has been the investigation of an enzyme capable of metabolizing GHB which is found in tissues such as liver and kidney as well as in brain. This enzyme, an NADP⁺-linked alcohol oxido-reductase which interconverts GHB and SSA, has been purified from hamster liver and partially purified from hamster brain. Throughout this report this enzyme will be referred to as GHB dehydrogenase so that the catalytic activity of interest to this laboratory will be clear. Present studies on this enzyme indicate that 1) it is present in most peripheral tissues as well as in brain, 2) it is an enzyme which is highly regulated, and 3) it appears to play an important role in the metabolism of GHB and, therefore, the regulation of tissue levels of GHB. The knowledge which we have gained from studies of the purified enzyme have allowed us to both design and interpret in vivo studies on factors which regulate tissue levels of GHB.

The finding that a number of biological intermediates, namely the transamination products of L-phenylalanine and of the branched-chain amino acids, are good inhibitors of the purified GHB dehydrogenase led to the prediction that these compounds might also inhibit the in vivo oxidation of GHB and thereby result in an increase in the tissue levels of GHB. This prediction has been tested. Measurement of tissue levels of GHB after infusion of α -ketoisocaproate (the transamination product of leucine) showed a twofold increase in kidney and

muscle but only a 23% increase in brain. Infusion of phenylacetate (a metabolite resulting from phenylalanine oxidation) gave a twofold increase of GHB in brain with a small decrease in the levels in kidney and muscle. This last effect was unexpected, and we are currently investigating the possibility that phenylacetate not only affects GHB dehydrogenase but also some enzyme in the biosynthetic pathway which functions in peripheral tissues. These studies are of significance since phenylacetate and α -ketoisocaproate are compounds which are known to accumulate in phenylketonuria and in maple sugar urine disease and link the metabolism of GHB to that of some of the amino acids.

Drugs such as salicylate and valproate are also good inhibitors of the purified enzyme. Valproate has already been shown by Snead et al., J. Neurochem. 19:47-52 (1980) to lead to increased brain levels of GHB. We have confirmed this observation and can now propose that the inhibition of GHB dehydrogenase is the cause of this elevation of the tissue level of GHB in brain. GHB-dehydrogenase has a much greater affinity for sodium valproate ($K_i=10^{-5}M$) than does either of the other enzymes involved in GABA or GHB metabolism (i.e., GABA-T, $K_i=10^{-4}M$ or SSA dehydrogenase, $K_i=10^{-3}M$). Recent studies in this laboratory have shown that salicylate (I.P.) will lead to a twofold increase in GHB levels in both brain and kidney. Again it is reasonable to propose that the increase tissue levels following administration result from the inhibition of GHB dehydrogenase and the decreased rate of degradation which would result.

Earlier work on the in vitro synthesis by GHB from GABA with intact brain mitochondria demonstrated that the rate of synthesis under anaerobic conditions was at least twice as fast as under aerobic conditions. When rats were exposed to 5.6% oxygen for a period of 2 hours, the concentration of GHB in whole brain was increased threefold and in kidney, twofold. The concentration in muscle was unaffected.

The effect of fasting was also investigated. Fasting decreased the tissue level of GHB in kidney over a period of three days and increased the level in muscle over the same period. Brain was unaffected. An unexpected finding was the interaction between fasting and low ambient O_2 . In the fasted animal exposure to low O_2 elevated the tissue level of GHB in muscle and prevented the increase seen in the kidney of the fed animal.

Although some of the changes in tissue levels can be explained on the basis of inhibition of the degradative enzyme and others on the change in the ratio of oxidized to reduced cofactors, it is apparent that these tissue levels must be regulated by many interacting factors. These in vivo studies are now completed and are being written up for publication.

Antibody.

In addition to the antibody which was raised in New Zealand white rabbits, we have also raised an antibody in a goat. This has enabled us to obtain much larger quantities of antiserum. We will continue to use this antibody in studies of the pathways of biosynthesis and degradation of GHB.

Precursor for GHB.

Efforts are currently underway to work out the biosynthetic pathway for GHB. We have used three main approaches to this problem. In the first, a number of carbohydrate intermediates, amino acids, and fatty acids were tested in an in vitro system for the ability to produce net synthesis of GHB. This has been a general survey of a number of possible precursors for GHB.

The second approach was similar to the first except that radioactive compounds were used in order to determine whether the compounds which gave rise to GHB in the first part actually contribute carbon atoms to the GHB molecule.

The third approach consists of in vivo experiments in which a radioactive precursor is injected and GHB is then isolated from the various tissues to determine whether the putative precursor has been incorporated in the GHB pool.

Results from the general survey of precursors, and preliminary work with radioactive compounds (in vitro), indicate that D,L- β -hydroxybutyrate and citrate are both capable of stimulating the formation of GHB, and of contributing carbon atoms to the skeleton of GHB. However, in the crude system of cell homogenate there is such a large amount of endogenous precursor or precursors that the specific activity of the GHB is reduced to about 10% of the specific activity of the precursor. The endogenous precursor is found in high concentration (up to 1 mMol/gr of kidney) in the soluble fraction of mitochondria from fed rats. Such high concentrations of endogenous precursor make it unlikely that β -hydroxybutyrate or citrate serve as the major precursor. While β -hydroxybutyrate does not appear to be a major precursor, its stimulating effect on the synthesis of GHB from endogenous precursors in the soluble mitochondrial fraction is quite marked.

A survey of organ systems other than brain has revealed that gamma-hydroxybutyrate is present in all organs in concentrations similar to that in brain, or in levels that are considerably higher than in brain. The kidney is particularly notable in that it has the highest concentration of gamma-hydroxybutyrate of the major organs, and it also has one of the highest concentrations of gamma-hydroxybutyrate dehydrogenase, the oxidoreductase which oxidizes gamma-hydroxybutyrate to succinic semialdehyde. The high level of this enzyme suggests that the rate of gamma-hydroxybutyrate synthesis may be rapid in the kidney. Such a rapid rate is surprising as glutamate decarboxylase is present in a 25-fold lower concentration than it is in brain.

A synthetic route for the formation of GABA from putrescine was demonstrated by Seiler who demonstrated that putrescine may be generated by the decarboxylation of l-ornithine or from the degradation of the larger polyamines, and may then be converted to GABA by two alternate pathways which have been shown to exist in liver and brain.

In the first pathway putrescine is acetylated in the nucleus and then is oxidatively deaminated by monoamine oxidase, and the resulting N-acetylated aminoaldehyde is further degraded to GABA.

In the second pathway putrescine is oxidatively deaminated by diamine oxidase to form the delta-1-pyrroline. The aminoaldehyde is then oxidized to GABA. *In vivo* inhibitor experiments were carried out to test the possibility that putrescine or ornithine are precursors of GHB in kidney. In one experiment the animals were treated with pargyline, a potent monoamine oxidase inhibitor which blocks the deamination of putrescine. In a second experiment animals were treated with difluoro-methyl-ornithine (DFMO), an irreversible ornithine decarboxylase inhibitor. The results of these treatments on tissue levels of GHB are shown below.

Treatment	GHB (nanomoles/g tissue)		
	Brain	Kidney	Muscle
Pargyline	2.45 ± 0.14 (7)	21.1 ± 2.5 (7)*	19.4 ± 2.8 (4)
Saline control	2.48 ± 0.45 (7)	31.3 ± 6.5 (7)	21.0 ± 5.4 (3)
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DFMO	3.01 ± 0.44 (4)	22.6 ± 3.7 (4)**	16.0 ± 2.2 (4)
Saline control	3.16 ± 0.41 (4)	42.2 ± 3.6 (4)	25.0 ± 3.4 (4)

* $p < 0.05$, ** $p < 0.01$, Mean ± SEM. Numbers of rats in parentheses.

Neither pargyline nor difluoromethyl-ornithine affected the concentration of gamma-hydroxybutyrate in the brain, a tissue where glutamate decarboxylase is exceptionally active, and no significant effect was seen in muscle. However, the gamma-hydroxybutyrate concentration was reduced by 33% in kidneys of animals which had been treated with pargyline, and by 46% in animals in which ornithine decarboxylase had been inhibited by difluoro-methyl-ornithine. Both effects were statistically significant.

Because inhibitors of putrescine metabolism were effective in lowering the concentration of gamma-hydroxybutyrate in the kidney, an attempt was made to determine which branch of the two degradative pathways for putrescine was involved (i.e., that going through N-acetyl putrescine or that through putrescine).

In an experiment in which [^{14}C] labeled monoacetyl putrescine was incubated with kidney homogenate and NADPH plus either NAD^+ or NADP^+ , no [^{14}C] could be found in the GHB which was isolated from the homogenate at the end of the incubation. When this experiment was repeated with [^{14}C]putrescine, the GHB isolated from the homogenate at the end of the experiment contained radioactivity which coincided exactly with the GHB peak on gas chromatography.

It is evident that putrescine does indeed contribute to the carbon skeleton of gamma-hydroxybutyrate. Data from the in vivo inhibitor study suggest that the contribution is quite large.

It should also be noted that kidney homogenate can decarboxylate glutamate to form GABA, but at present we do not have enough data from animals treated with glutamate decarboxylase inhibitors to judge the importance of glutamate as a precursor for gamma-hydroxybutyrate synthesis in vivo.

Finally, it should also be noted that while all of gamma-hydroxybutyrate synthesis from GABA can be inhibited in vitro by means of agents such as hydrazine sulphate and aminoxy acetic acid, gamma-hydroxybutyrate synthesis from endogenous substrate in kidney is only 80% inhibited, which suggests the possibility of another synthetic route which does not involve GABA.

Thus far it appears that the major portion of GHB is derived from GABA. In brain, GABA is formed from glutamate through the GABA shunt pathway; in other tissues preliminary evidence suggests that polyamines or arginine may serve as the GABA precursor. The synthesis thus may be linked to the citric acid cycle, to the urea cycle, and to the polyamines which appear to play some regulatory function within the cell nucleus. The degradation of GHB is most likely accomplished by glucuronate reductase under circumstances which require a stoichiometric oxidation of aldehydes such as D-glucuronate to occur. Furthermore, the degradation of GHB appears to be controlled by several naturally-occurring α -keto acids, and phenyl ethyl alcohols which have recently been shown to inhibit D-glucuronate reductase. Pharmacological levels of GHB have been shown in this laboratory to produce a striking reduction of glucose utilization by the brain. Taken together these findings suggest that GHB might serve as some form of intracellular messenger, perhaps linking both protein and carbohydrate metabolism with nuclear events.

Effect of Naloxone on the Pharmacological Action of GHB.

It has been reported that naloxone can reverse some of the pharmacological effects of GHB, i.e., the effect on EEG charges, the accumulation of dopamine in the nigrostriatal pathway and the behavioral effect. Investigations in this laboratory of naloxone as an antagonist of GHB revealed that the effect of GHB on cerebral glucose utilization could be partially reversed in selected regions of the central nervous system. It was also observed that naloxone affected the drop in body temperature caused by a pharmacological dose of GHB. This aspect of the GHB project has been completed and a report of this work is published.

Significance to Biomedical Research and Program of Institute.

The studies described in this project coincide with one of the dominant interests of this laboratory, i.e., the study of the many factors which control cerebral metabolism. The effect of γ -hydroxybutyrate on cerebral glucose utilization was discovered in this laboratory and served as an impetus for the studies which have followed. γ -Hydroxybutyrate, a normal constituent of mammalian brain, has been implicated in control of dopaminergic functions, in seizure states, anesthesia and temperature regulation. Many of its actions bear

a strong resemblance to those of the opiates and like the opiates can be antagonized by naloxone. Recently, two mentally and physically retarded patients with neurological abnormalities and with a γ -hydroxybutyric aciduria have been reported.

The clarification of the origin, disposition, and function of γ -hydroxybutyrate should serve to enlarge our knowledge of normal and abnormal functions and biochemistry in the brain.

Proposed Course.

1) Studies on the biosynthetic pathways for GHB will continue. This will include identification of new pathways in peripheral tissues, elucidation of the steps in these pathways, and finally a study of factors which control these pathways.

2) The collaborative study to search for patients with a defect in GHB metabolism will continue.

3) We expect to initiate a collaborative study on the possible rate of GHB dehydrogenase in animals with narcolepsy.

4) Studies to clarify the effects of GHB at the cellular level will be initiated. These will include the effect of GHB on ion transport, on the release of calmodulin, on ATP'ases, and on protein synthesis.

The project which was designed to investigate the interrelationship of ketone bodies on the synthesis of high energy intermediates such as GTP and cyclic GMP in the central nervous system has been terminated due to the death of one of the principal investigators, Dr. Thomas Duffy. This project was designated Project #2, The Effect of Ketones on Cerebral Metabolism, in the previous annual report.

The work on the incorporation of 2-deoxyglucose into glycogen has been completed; the manuscript has been written and accepted for publication in the Journal of Neurochemistry. This work was designated Project #3, The Incorporation of 2-Deoxyglucose into Glycogen, in the previous annual report.

Publications:

Crosby, G., Ito, M., Kaufman, E., Nelson, T., and Sokoloff, L: Naloxone pretreatment alters the local cerebral metabolic effect of γ -hydroxybutyrate in rats. Brain Res. 275: 194-197, 1983.

Nelson, T., Kaufman, E.E., and Sokoloff, L.: 2-Deoxyglucose incorporation into rat brain glycogen during measurement of local cerebral glucose utilization by the 2-deoxyglucose method. J. Neurochem., in press, 1984.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00882-17 LCM

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Studies on Regional Cerebral Circulation and Metabolism

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I. Louis Sokoloff Chief, Lab. Cerebral Metabolism LCM, NIMH

Others: Charles Kennedy Guest Researcher LCM, NIMH
 Massako Kadekaro Visiting Scientist LCM, NIMH
 Astrid Nehlig Visiting Associate LCM, NIMH
 Thomas Nelson Senior Staff Fellow LCM, NIMH
 Linda Porrino Senior Staff Fellow LCM, NIMH
 Carolyn B. Smith Senior Staff Fellow LCM, NIMH
 Ralph U. Esposito Senior Staff Fellow LCM, NIMH
 Giovanni Lucignani Visiting Fellow LCM, NIMH

COOPERATING UNITS (if any)

Theoretical Statistics and Mathematics Branch, B, NIMH (Patlak, C. and Pettigrew, K.); NINCDS, NIH (Burns, R. and Kopin, I.); Maryland Psychiatric Research Center, Univ. of Md., Dept. of Psychiatry, Baltimore, MD (Tamminga, C.)

LAB/BRANCH

Laboratory of Cerebral Metabolism

SECTION

Developmental Neurochemistry Section

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

12.0

PROFESSIONAL:

5.5

OTHER:

6.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A method has been developed for the quantitative determination of the rates of local glucose consumption in the discrete functional and structural components of the brain in conscious or anesthetized laboratory animals. The method is based on the use of [14 C]deoxyglucose as a tracer for glucose flux through the hexokinase step. Local [14 C]deoxyglucose-6-phosphate concentrations in the tissues of the CNS are measured by a quantitative autoradiographic method. Inasmuch as the autoradiographs of the relative rates of local glucose consumption can be used directly for mapping metabolically, and therefore functionally, linked structures in the CNS, the method is being used to study alterations in the energy metabolism of the discrete functional and structural components of the brain in a variety of physiological, pharmacological, and pathological states.

Previous work in this Laboratory led to the development of a method for the measurement of the rates of blood flow in the structural and functional units of brain in conscious laboratory animals. The method was based on the uptake of a radioactive, chemically inert gas into the tissues of the brain, and a unique quantitative autoradiographic technique was developed which made possible the measurement by densitometric procedures of the concentrations of the radioactive tracer in the individual structures of the brain down to a resolution of 0.2-0.5 millimeters. The key to the fine resolution of the method was the autoradiographic technique.

Although measurement of local cerebral blood flow is inherently interesting with respect to the physiology, pharmacology, and pathology of the circulatory system, it is of limited value in studies of cerebral functional and biochemical activity. The Laboratory, therefore, addressed itself to the development of a method to measure local cerebral energy metabolism with the same degree of structural resolution because energy metabolism could be expected to relate more closely to local cerebral functional activity. It was always anticipated that the quantitative autoradiographic technique designed for the blood flow method would also be at the heart of such a method. It was necessary, however, to choose an appropriately labeled precursor of cerebral energy metabolism. Oxygen could not be used because there are no suitable radioisotopes of oxygen. [^{18}O]Glucose also appeared to be unsuitable because glucose is too rapidly metabolized, and its radioactive products are too quickly removed from brain. It was, therefore, decided to use [^{14}C]deoxyglucose, an analogue of glucose which is handled qualitatively just like glucose by the transport system in the blood-brain barrier and by the initial enzyme, hexokinase, in the pathway of glucose metabolism. Once phosphorylated, however, the deoxyglucose is trapped, unlike glucose which is metabolized further to carbon dioxide and water. Quantitatively, however, deoxyglucose phosphorylation and glucose phosphorylation or utilization are different inasmuch as the transport carrier and the enzyme discriminate kinetically between the two substrates. It appeared to be a simple matter to apply the autoradiographic technique to measure deoxyglucose phosphorylation, but to relate it to the steady state rate of glucose flux through the phosphorylation step, which is a measure of the rate of glucose consumption, required the solution of numerous theoretical and technical problems.

A theoretical model, which encompassed all that we knew about deoxyglucose and glucose transport between brain and blood and their metabolism in brain tissue, was constructed, and mathematical relationships to describe the model were developed. Experiments were done on one point or another to evaluate and, if necessary, to revise the model and the mathematical relationships to fit the model closer to the real situation.

It was clear from the model that to determine the rate of glucose consumption from the rate of [^{14}C]deoxyglucose phosphorylation would require the determination of the distribution volumes of deoxyglucose and glucose in the cerebral tissues and the hexokinase kinetic constants (V_{max} and K_m) for both deoxyglucose and glucose. By appropriate mathematical manipulations, it was possible to segregate all these separate constants into a single "lumped constant" encompassing all of them. It was now necessary to determine only the single lumped constant rather than the six individual ones. Further mathematical analyses revealed the way to

design an experiment to determine the "lumped constant". Another equation was developed from the model which showed that if the arterial concentration was maintained constant for a sufficient length of time, e.g., at least 20 minutes, then the ratio of the cerebral extractions of deoxyglucose and glucose would reach a constant level equal to the lumped constant. With the help of the Theoretical Statistics and Mathematics Branch, it was found possible to derive from the analyses of plasma disappearance curves of deoxyglucose an intravenous infusion schedule which results in a constant arterial deoxyglucose concentration for up to 45 minutes or longer. Surgical procedures were developed in the rat, monkey, and cat to sample arterial and cerebral venous blood from which the extraction ratios are determined. The lumped constant has been fully determined in the conscious and anesthetized rat; its value is 0.48, and it is unchanged in a variety of physiological and pharmacological states. The lumped constant has recently been determined in the monkey and the cat; the values have been found to be 0.344 and 0.41, respectively. In collaboration with T. Duffy of the Department of Neurology, Cornell University, the lumped constant has been measured in the dog and found to be 0.56. In a collaboration with Dr. Robert Abrams at the University of Florida it has been measured in fetal and neonatal sheep. The values in pre- and postnatal life were virtually identical: 0.40.

All the theoretical and technical problems were solved, and the method has now been completely operative for the last seven years. An equation has been derived which relates the rate of glucose consumption to measurable variables and allows the calculation of glucose consumption in the discrete structural and functional units of the brain. The equation prescribes the procedure to be used and the variables to be measured. An intravenous pulse of [¹⁴C]deoxyglucose is injected, and arterial plasma concentrations of [¹⁴C]deoxyglucose and glucose are measured from the time of injection until 30-45 minutes when the animal is decapitated, and the head frozen. Sections of brain are prepared from which local cerebral tissue [¹⁴C]deoxyglucose concentrations are determined by the quantitative autoradiographic technique. From these measured variables and the lumped constant, local cerebral glucose utilization is calculated by the equation. The procedure for calculation has been programmed, and all the calculations are carried out by a computer. The method has now been in use for at least seven years in this Laboratory and in laboratories around the world. It has been found to be generally successful but requires adaptation to certain types of pathophysiological states, such as severe hypoglycemia, status epilepticus, and ischemia, when the balance between glucose supply and glucose consumption in brain is disturbed. In these cases, the method is still valid, but the rate constants and lumped constant (components of the operational equation) have to be recalibrated for the specific condition.

Numerous applications of the method to physiological and pharmacological conditions have been made in this Laboratory and published in recent years. Much of the Laboratory's program has been devoted to such applications, including studies during the last year.

In a previous study in this Laboratory Dr. Linda J. Porrino demonstrated that the psychostimulant, amphetamine, activated glucose utilization in the components of the dopaminergic pathways of the extrapyramidal motor system at high doses and components of the dopaminergic mesolimbic system at lower doses.

These changes were found to correlate with the different patterns of behavior observed at high and low doses. The behavioral effects of methylphenidate, another psychostimulant are similar to those of amphetamine, but its biochemical actions are distinguishable from those of amphetamine. Reserpine pretreatment blocks methylphenidate elicited behaviors and dopamine release but has no effect on amphetamine elicited behaviors or release. Dr. Porrino and Dr. Giovanni Lucignani compared the effects of acute methylphenidate administration on local cerebral glucose utilization to those seen with amphetamine. Significant dose related increases in LCGU were observed within the extrapyramidal dopaminergic system, particularly in the subthalamic nucleus. In dopaminergically innervated cortex and in the nucleus accumbens increases in glucose utilization were seen at low doses, but not at high doses. Despite differences in their biochemical actions, then, the pattern of glucose utilization following methylphenidate administration is similar to that seen with amphetamine, particularly with regard to the nucleus accumbens and the extrapyramidal system. Similar behavioral patterns elicited by both methylphenidate and amphetamine have been found therefore to correlate with similar specific changes in brain energy metabolism.

Recently, an animal model of Parkinson's disease has been developed by administration of N-methyl-4-phenyl-1236 tetra-hydro-pyridene (MPTP) to primates. This drug specifically destroys the cells of the substantia nigra pars compacta, and the monkeys have all the major clinical features of Parkinson's disease in humans. Dr. Linda Porrino in collaboration with Dr. Richard Burns and Dr. Irwin Kopin (NINCDS) have applied the deoxyglucose method to monkeys treated with MPTP and to MPTP-treated monkeys receiving L-DOPA therapy. Glucose utilization in monkeys made Parkinsonian when compared to glucose utilization in normal monkeys was found to be altered in some areas of the basal ganglia, most prominently in the substantia nigra pars compacta where dopamine cells had been destroyed as well as in the subthalamic nucleus and the external segment of the globus pallidus. No changes were found in dopaminergically innervated limbic areas such as the nucleus accumbens or lateral septum, or in thalamic and cortical regions. Glucose utilization in monkeys receiving L-DOPA therapy following MPTP treatment was increased throughout areas of motor and premotor cortex as well as in motor nuclei of the thalamus, ventral anterior and ventrolateral nuclei. The most prominent increases were found in the subthalamic nucleus and in the globus pallidus. These changes in glucose utilization are in sharp contrast to the lack of effects on energy metabolism seen following the administration of L-DOPA to normal animals. These results are significant for two reasons. First, they place emphasis on the role of the globus pallidus-subthalamic nucleus circuit in the organization and performance of normal movement. Second, these results provide insights into the therapeutic actions of L-DOPA in Parkinson's disease, by clearly demonstrating the differences in this drug's actions in normal and diseased brain.

Further work on this collaborative project will concentrate on the role of the subthalamic nucleus in movement disorders and on the modes of action of other therapeutic agents used in the treatment of Parkinson's disease.

In another study Dr. Astrid Nehlig and Dr. Linda Porrino have studied brain energy metabolism during the estrus cycle in female rats. Glucose utilization in the brain of female rats displayed cyclic variation with the highest levels

evident during proestrus and metestrus stages of the cycle. Significant changes were found in the hypothalamus, particularly in the preoptic and arcuate areas and in some limbic structures. Comparison of the values obtained in females to those seen in males revealed significant differences in rates of glucose utilization in areas known to be involved in control of sexual behavior. Rates in most structures, however, did not differ in males and females.

The gut peptide cholecystokinin (CCK) recently identified in brain has been examined for its effect on local cerebral glucose utilization. CCK has a widespread distribution in brain with the highest levels found in cortex, hippocampus, hypothalamus, striatum and amygdala, and its peripheral administration results in satiety as well as reductions in locomotor activity. There is some question, however, as to whether CCK can enter brain and act therein after peripheral administration. Dr. Giovanni Lucignani along with Dr. Linda Porrino and Dr. Carol Tamminga (University of Maryland, Psychiatric Research Center) have shown with the deoxyglucose method that specific changes in the rates of glucose utilization are produced in brain by peripheral CCK administration. Decreases were seen in cerebral cortex, substantia nigra and ventral tegmental area, lateral habenula and the nucleus of the solitary tract. These changes are concordant with the known effects of CCK on dopaminergic transmission and its role in the regulation of food intake.

The deoxyglucose method measures the amount of 2-deoxyglucose-6-phosphate which is formed over a period of 45 minutes following an injection of 2-deoxyglucose. The rate at which this analogue accumulates is related to the rate of glucose metabolism during that interval. The method has been challenged in some circles since its publication in 1977 on the grounds that it fails to account for loss of label as a result of the action of glucose-6-phosphatase, an enzyme generally considered to be absent or negligible in brain. Underestimation of the activity of this enzyme would result in values for glucose utilization which would be too low. Despite the close agreement between values for glucose metabolism of the whole brain obtained by the standard Kety-Schmidt method and the deoxyglucose method, the controversy over the effect of glucose-6-phosphatase in the brain continues. In view of the potential impact that these recurrent criticisms may have on the use of the method, it has been necessary to undertake a detailed experimental investigation of the validity of these criticisms.

Two papers critical of the deoxyglucose method have been published recently. The first paper by Huang and Veech, *J. Biol. Chem.*, 257:11358-11363 (1982), purported to demonstrate that brain dephosphorylates glucose-6-phosphate at a rate equal to 35% of the rate of glucose phosphorylation. In their method a mixture of uniformly labeled [^{14}C]glucose and [2- ^3H]glucose was given as a pulse into the external carotid artery of rats, and at various times after the pulse, brains were obtained by the freeze-blowing technique and analyzed for the $^3\text{H}/^{14}\text{C}$ ratio in the glucose fraction. Inasmuch as tritium or hydrogen on the second carbon of glucose-6-phosphate is released and exchanges with hydrogen from water during isomerization of glucose-6-phosphate to fructose-6-phosphate, a greater rate of loss of tritium than ^{14}C in the brain glucose pool should be evidence that some of the glucose is reformed by the dephosphorylation of glucose-6-phosphate which itself is in equilibrium with fructose-6-phosphate.

The rate of dephosphorylation of glucose-6-phosphate found by these workers was almost equal to that of liver and suggests that brain and liver should have similar glucose-6-phosphatase activities. This conclusion is at variance with all previous biochemical experience; previous measurements have shown rates of 17 $\mu\text{moles/g/min}$ in liver and 0.06 $\mu\text{moles/g/min}$ in brain (Hers, 1957). Furthermore, in human glycogen storage disease, type 1, in which glucose-6-phosphatase is genetically absent, patients have massive hepatomegaly which results from the conversion of the glucose-6-phosphate, in excess of metabolic requirements, into glycogen rather than into glucose which can be exported from the liver. No such biochemical changes are noted in brain, indicating that brain glucose-6-phosphatase must not normally play a significant role in brain.

If the Huang and Veech observations were correct, a substantial correction factor (k_4) would be required in the operational equation of the 2-deoxyglucose method. The model used in the deoxyglucose method could easily be altered to accommodate k_4 , the rate of dephosphorylation, if there were a significant phosphatase activity in brain. However, biochemical evidence accumulated over thirty years suggests that glucose-6-phosphatase is very inactive in brain.

A second paper by Sacks et al., *Neurochem. Res.* 8:661-685 (1983), presents experimental data from which the authors claim to prove that the deoxyglucose method is wrong. Three different experiments are described which supposedly show that: 1) there is no increase in 2-deoxyglucose concentration with time. (The continued increase in 2-deoxyglucose-6-phosphate in brain is a mandatory requirement of the 2-deoxyglucose method.) 2) Four minutes after a pulse of a mixture of 2-[1- ^{14}C]deoxyglucose and [1- ^3H]glucose, there is a net loss of 2-deoxyglucose from the brain while there is no net loss of glucose into the venous blood. 3) [^{14}C]glucose-6-phosphate is taken up by the brain, where it is dephosphorylated and returned to the cerebral venous blood as free glucose.

In response to these criticisms, the Laboratory of Cerebral Metabolism has invested a year of research effort in repeating these experiments. Experiments have been performed which show that all of the assertions from the Sacks paper listed above are completely groundless.

2-deoxyglucose was administered to groups of rats, and brains were obtained by means of the freeze-blowing technique at time intervals ranging from 2 to 45 minutes after the pulse. Plasma arterial samples were also collected. The concentration of 2-deoxyglucose-6-phosphate increases rapidly in brain during the first 5 minutes after the pulse, and thereafter continues to rise more slowly as the plasma 2-deoxyglucose level falls to low levels. The measured concentration of 2-deoxyglucose-6-phosphate agrees well with that predicted by the theoretical model and equation.

The second point made by the Sacks paper is that 2-deoxyglucose and glucose do not behave alike; i.e., 2-[^{14}C]deoxyglucose diffuses out of the brain while glucose does not, after infusing a pulse of a mixture of these two substances into rats. This finding is completely predictable and is to be expected given what is known about the rates of transfer of glucose and 2-deoxyglucose between brain and blood. The metabolic rate constant (k_3) is three-fold higher for glucose than for its analogue, and results in such a rapid phosphorylation of

glucose that the tissue concentration of [^{14}C]glucose is kept too low to provide a sufficient driving force for net diffusion back to the plasma after termination of the pulse. 2-deoxyglucose is metabolized more slowly and remains in substantial concentration in the brain after the pulse. Some of this free 2-deoxyglucose then diffuses back into the plasma when its concentration in brain exceeds that in plasma. This is entirely predicted by the model and equation of the deoxyglucose method.

The third point in Sacks' paper is experimentally invalid. It is well known that glucose-6-phosphate cannot cross the blood-brain barrier and enter the brain from the blood. This has been proved by infusing [^{32}P]glucose-6-phosphate into rats and measuring the amounts of label trapped in the brain. In all cases the amount of label trapped in the brain was approximately 2% of the concentration present in the blood, which is the amount contributed by the amount of blood in the brain. Furthermore, it has been demonstrated that there is no significant A-V difference for organic phosphate across the brain during a steady state during infusion of [^{32}P]glucose-6-phosphate. An artificial, apparent A-V difference can be demonstrated in animals in a non-steady state which is true with any tracer, even Evans Blue dye which is a non-diffusible reference substance.

Examining Sacks' criticisms, therefore, shows that: 1) Free 2-deoxyglucose in brain falls with time after the pulse and that the product, 2-deoxyglucose-6-phosphate accumulates at a rate which is in good agreement with the theoretical amount predicted by the 2-deoxyglucose method. 2) Simulations using the appropriate experimentally measured rate constants for glucose and 2-deoxyglucose predict exactly the kinetic behavior noted by Sacks in the cerebral arterial and venous plasma. A negative V-A difference for 2-deoxyglucose eventually appears after the arterial concentration has fallen low enough while glucose is still being taken up by the brain. 3) The experiment in which glucose-6-phosphate was infused and glucose concentrations measured in cerebral arterial and venous plasma was misinterpreted by Sacks. There is no uptake of glucose-6-phosphate by brain and, therefore, no possibility of its dephosphorylation by brain glucose-6-phosphatase activity.

Experiments are currently underway to examine the validity of the report by Huang and Veech that 35% of the glucose-6-phosphate formed by the hexokinase catalyzed phosphorylation of glucose is hydrolyzed by glucose-6-phosphatase. The experiment reported by these workers has been repeated, but in rats in which the cerebral circulation is kept intact as it is in the usual application of the 2-deoxyglucose method. In a group of eight rats spanning the interval 2 to 7 1/2 minutes, there was no decline in the $^3\text{H}/^{14}\text{C}$ ratio of brain glucose with time in direct contradiction of the reported observations of Huang and Veech. The difference may be due to the fact that Huang and Veech obstructed the carotid circulation of the rats bilaterally for 24 hours prior to the experiment. This produced brain damage.

Other rats have therefore been prepared with the carotid artery obstruction like that produced by Huang and Veech. Many of these animals have obvious neurological deficits. Brains and plasma samples from these rats are being assayed for $^3\text{H}/^{14}\text{C}$ glucose ratios to see if the Huang and Veech findings could have been caused by the effects of severe cerebral ischemia.

The attempts to replicate the Huang and Veech experiment and the Sacks experiments have shown that there is no evidence for brain glucose-6-phosphatase activity within the 45 minute following a pulse of 2-deoxyglucose. Furthermore, they demonstrate that the phosphorylated product of 2-deoxyglucose increases with time after a pulse of labeled 2-deoxyglucose exactly as described by the deoxyglucose method, supporting the validity of the model on which it is based. The 2-deoxyglucose method is no less valid than when it was first described. There is no reason to alter the operational equation to correct for cerebral glucose-6-phosphatase activity because there is no dephosphorylation of glucose which occurs in the first 45 minutes after an intravenous pulse of 2-deoxyglucose.

Significance to Biomedical Research and Program of Institute.

The deoxyglucose method has made it possible for the first time to measure the rates of glucose utilization simultaneously in all functional and structural components of the central nervous system of conscious, behaving animals and now also in man. Because the method was developed in our Laboratory, it has been our responsibility to survey its applicability to the various types of conditions in which it might be applicable. The program has, therefore, been somewhat heterogeneous covering a wide range of physiological, pharmacological, pathological, and altered behavioral states. The method and its wide-ranging usefulness has now been more or less established, and it is used extensively throughout the world in neuroanatomical, neurophysiological, neuropharmacological, psychiatric, neurological, and neurosurgical research, and its wide acceptance is directly related to the results of studies in this project.

Proposed Course.

Applications of the deoxyglucose method to problems of neurophysiology, neuropharmacology, and neurology will be continued, either by completion of ongoing projects or the initiation of new ones.

The biochemical studies that already appear to refute the criticisms of the deoxyglucose method will be completed and published in appropriate biochemical and neurochemical journals.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
 Z01 MH 00887-07 LCM

PERIOD COVERED
 October 1, 1983 through September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
 The Extended Visual System of the Macaque Monkey

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Charles Kennedy	Guest Researcher	LCM NIMH
Other:	Louis Sokoloff	Chief, Lab. Cerebral Metabolism	LCM NIMH
	Masanori Ito	Visiting Associate	LCM NIMH
	Mortimer Mishkin	Chief, Lab. Neuropsychology	LN NIMH
	Kathleen Macko	Staff Fellow	LN NIMH
	Jocelyn Bachevalier	Visiting Associate	LN NIMH

COOPERATING UNITS (if any)
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LAB/BRANCH
 Laboratory of Cerebral Metabolism

SECTION
 Section on Developmental Neurochemistry

INSTITUTE AND LOCATION
 NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.4	0.2	0.2

CHECK APPROPRIATE BOX(ES)

(a) Human subjects (b) Human tissues (c) Neither

(a1) Minors

(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The deoxyglucose method is being applied to the monkey to advance knowledge regarding the parts of the brain which are involved in the process of visual information. By measuring rates of local cerebral glucose utilization in animals during their performance of tasks involving different types of visual stimuli we anticipate learning which parts of brain are involved in such functions as discrimination, memory and motivation. Also by studying animals at various ages, information will be obtained regarding the maturation of the visual processing system.

Project Description:

The goal of the collaborative effort initiated in 1978 was to map regions of monkey brain which were responsive to visual stimulation. The deoxyglucose method has been shown to be sensitive to even small differences in functional activity, and it was hoped that it might be possible to shed light on such complex aspects of visual function as discrimination, memory, or even the mechanism by which the brain assigns a value judgment on the character of visual stimulation and then initiates a response to it. The procedure followed is to prepare animals so that one hemisphere is completely deprived of visual input. One optic tract is sectioned as is the corpus callosum and forebrain commissures. Because the intact brain functions symmetrically and therefore has equal metabolic rates in all homologous structures, the finding of right-left differences in metabolic rates in the surgically prepared animals serves to identify the visually responsive regions. The experiments to date have demonstrated that these include the striate cortex and entire expanse of the circumstriate and inferior temporal cortex as far forward as the temporal pole.

The cortical areas related to vision were found to include the entire expanse of striate, prestriate, and inferior temporal cortex as far forward as the temporal pole, the posterior part of the inferior parietal lobule, and the prearcuate and inferior prefrontal cortex; subcortically, in addition to the dorsal lateral geniculate nucleus and superficial layers of the superior colliculus, the structures related to vision included large parts of the pulvinar, caudate, putamen, claustrum, and amygdala. These results, which are consonant with a model of visual function that postulates an occipito-temporo-prefrontal pathway for object vision and an occipito-parieto-prefrontal pathway for spatial vision, reveal the full extent of those pathways and localize their points of contact with limbic, striatal, and diencephalic structures.

A major project has been the delineation of the exact border between visual and non-visual cortex throughout this extended region. This has been facilitated by the computer-assisted image-processing system which makes possible the estimation of average values for histologically distinct cortical areas. The border separating visual from non-visual cortex has now been mapped in detail through the entire extent of striate cortex (Areas OB and OA) to the inferior convexity of the temporal lobe (Areas TEO and TE).

In other experiments the contribution of the commissural systems to these visually responsive cortical areas was determined. The commissural systems are those which transmit visual information from cortex across the mid-line to contralateral cortex. This was done by comparing average rates of glucose utilization in cortex in monkeys which had the optic tract alone sectioned with those which had had optic tract section plus commissural section. The results indicated that the commissural contribution is very largely due to a region designated TE in the anterior portion of the inferior temporal lobe.

These findings gave no explanation, however, for the results of anatomic investigations which indicate that there is a much wider distribution of commissural fiber projections to the visual cortical pathway. A possible

explanation for the failure of a metabolic response to occur over the full extent of these projections is that the cortical cells must be continually activated by an intact retino-geniculato-cortical pathway.

To test this hypothesis monkeys were prepared by "blinding" the right hemisphere in a manner different from that employed previously, namely, optic tract section. Instead a longitudinal cut was made in the optic chiasm together with occlusion of the right eye. This preserved the anatomic continuity of right sided retinal innervation to the cortex while depriving it of stimulation. Through a comparison of glucose utilization in the right hemispheres of these animals and of those studied previously with right optic tract section, the functional effectiveness of commissural input with and without spontaneous retinal input could be evaluated. A finding of a higher level of metabolic activity throughout the inferior temporal cortex of the former would indicate that the commissural fibers do require the intact innervation from the retina in order to respond. A preliminary analysis shows that there is no consistent difference between the visual cortical metabolic rates of animals with the optic tract cut and those prepared with the chiasm cut and eye occluded. Thus any commissural contribution to vision in the prestriate-posterior temporal region from an intact retina appears not detectable in the limited number of studies analyzed to date. To explain this apparent metabolic inertia of an anatomically established pathway may require another experimental approach.

The functional development of the extended cortical visual system is being studied by preparing infant monkeys with unilateral optic tract section and forebrain commissurotomy. The deoxyglucose method is then employed in the manner described for the mature animals. From a knowledge of the maturational characteristic of visual function the age range for these experiments was chosen to be 1 day to 5 months. The results show that there are systematic, age-related changes in rates of glucose utilization in normal visual related cortex as well as right-left differences between intact and visually deprived cortex. In all cortical visual areas of the intact hemisphere glucose utilization was lowest in the youngest subjects and reaches a maximum at four months. A single study at six months of age is at adult levels suggesting that the lower, mature rate is achieved long before pubescence. As in adult monkeys, the intact hemisphere of infants shows a progressive decline in glucose utilization along the ventral cortical visual pathway. This gradient was seen in all animals but was shallowest in the two youngest. The deprived hemisphere had low rates of glucose utilization compared to the intact hemisphere at all ages. The differences were greatest in the primary visual cortex (area OC) and smallest in the most anterior temporal cortex (area TE). These differences varied systematically with the age of the animal with maturation being accompanied by a serial increase of the intact-deprived difference until four months of age when it reached a maximum. This age of the peaking of both the absolute rate in the intact cortex and the intact-deprived difference corresponds with the age when the animal attains the capacity for object recognition (Bachevalier and Mishkin, *Int. J. Psychophysiol.*, 1983).

In the experiments cited above many animals had been trained to respond to a specific visual stimulus with unimanual key-pressing to obtain a water reward. Thus the same experiments which were used to map the extended visual system also

provided information on the metabolic responses to motor activity. While the motor pathways of the brain have been identified by other techniques, and thus are known, these experiments served to delineate the specific subdivisions of many structures which selectively are activated in the unimanual key-pressing. They provided new information on somatotopic localization of arm-hand movements. This was particularly well-defined in the study of cerebellar cortex. A large part of Crus II of the ipsilateral cerebellar cortex was shown to be selectively responsive in the animals' performance of the task. Also participating, but with a lesser percent change, was the lateral portion of the vermis in lobules III-VI. Localized increments in the rate of glucose utilization were also noted in VL and VPL of the thalamus, part of the globus pallidus and discrete zones of cerebral cortex (S, S_{II}, M) and a part of the supplementary motor area. A noteworthy feature of this mapping study of motor activity is that a much greater metabolic increment was found in structure concerned with sensory monitoring of motor activity than in those related to the motor activity itself.

The results during the last year are the same, and the only difference is that an additional number of animals have been added to the series. The slowness of the project is due to the availability of animals, the training of the animals, and the time it takes to analyze the results of the experiments.

Significance to Biomedical Research and to Program of the Institute.

This project represents a collaborative effort between the Laboratory of Cerebral Metabolism and the Laboratory of Neuropsychology in which the specialized expertise of each Laboratory is brought to bear on the use of the deoxyglucose method to study higher nervous functions, in this case the higher level processing of visual information beyond the primary visual system. The advantage of this approach is the ability to examine all local regions of the brain simultaneously in unanesthetized animals. It is hoped that these studies will help to elucidate the regions of the brain involved in integrating sensory inputs and eliciting appropriate affective responses.

Proposed Course.

The analysis of data obtained in experiments already carried out will be continued. To establish age related changes accompanying maturation more monkeys will be added to the series. These will be 3-6 months of age. Yet to be undertaken are experiments with different types of visual stimulation and with visual stimulation associated with tasks requiring learning and memory. Additional animals will be added to the awake and sleep series.

The Laboratory of Neuropsychology is reporting on this project with Report No. Z01 MH 02033-07 LN, titled "Functional Mapping of Sensory Systems".

Publications:

Reported in Z01 MH 02033-07 LN.

Kennedy, C.: Metabolic mapping of the primary visual pathway. In Sokoloff, L. (Ed.): Brain Imaging and Brain Function. New York, Raven Press, 1984, in press.

Macko, K.A., Bachevalier, J., Kennedy, C., Suda, S., Sokoloff, L., and Mishkin, M.: Functional development of the ventral cortical visual pathway measured by the 2-deoxyglucose method. Soc. Neurosci. Abstr. 9: 375, 1983.

Macko, K.A. and Mishkin, M.: Metabolic mapping of higher order visual areas in the monkey. In Sokoloff, L. (Ed.): Brain Imaging and Brain Function. New York, Raven Press, 1984, in press.

Sokoloff, L. (Ed.): Brain Imaging and Brain Function. New York, Raven Press, 1984, in press.

PERIOD COVERED
 October 1, 1983 through September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
 A Method for the Determination of Local Rates of Protein Synthesis in Brain

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)
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 Lab. of Neuropsychology, NIMH; University of Michigan, Ann Arbor, MI (B.W. Agranoff); Washington University, St. Louis, MO (Robert Collins).

LAB/BRANCH
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SECTION
 Developmental Neurochemistry Section

INSTITUTE AND LOCATION
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TOTAL MAN-YEARS	PROFESSIONAL	OTHER
6.0	4.0	2.0

CHECK APPROPRIATE BOX(ES)
 (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A method is being developed for the estimation of local rates of protein synthesis in brain in vivo. The method is based on the use of L-[1-¹⁴C]leucine as a tracer for the incorporation of leucine into protein. Six kinetic models for the behavior of leucine on brain have been designed. By mathematical analysis of the kinetics of exchange of the amino acid between plasma and the tissue pool(s) and its incorporation into protein, equations have been derived for each model that define the rate of amino acid incorporation into protein in terms of the time course of plasma-specific activity, final tissue concentration of ¹⁴C, and experimentally determined kinetic constants. Tissue concentrations of ¹⁴C are determined by quantitative autoradiography. Experiments are being carried out to test the validity of the various models.

The method is currently being applied to studies of aging, development, hypothyroidism, regeneration, and sleep.

Project Description:

A method is being developed for the estimation of local rates of protein synthesis in brain in vivo. This method is similar to the [^{14}C]deoxyglucose method in that it is based on enzyme kinetic principles as applied to the measurement of reaction rates in vivo with labeled tracers as substrates. In order to measure the rate of the reaction, one must know the amount of labeled product formed in a given interval of time and the integrated specific activity of the precursor. In an in vivo experiment the precursor pool cannot be sampled and the specific activity determined directly. It is necessary, therefore, to design a model for the behavior of the precursor in vivo and by kinetic analysis of the model to derive a relationship between the entire history of the precursor specific activity in the plasma (which can be sampled and measured directly), the integrated specific activity of the precursor pool in the tissue, and the rate of the reaction. Six kinetic models with progressively increasing complexity to take as many of the processes and factors into account as possible have been developed. By mathematical analysis of the kinetics of exchange of the amino acid between plasma and tissue and its incorporation into protein an operational equation for each model has been derived. Studies are in progress to identify the simplest model that adequately describes the processes proceeding in vivo.

For all of the models we have chosen L-[1- ^{14}C]leucine as the radiolabeled tracer for this method because the $^{14}\text{CO}_2$ derived from its metabolism is rapidly diluted in the pool of CO_2 and cleared from the tissue. There are, therefore, no side-reactions with radioactive products other than the labeled protein. Our current and most comprehensive model (Model VI) for the behavior of leucine in brain includes an extracellular and two intracellular compartments. The intracellular compartments are the precursor pool for protein synthesis, consisting of the activated amino acid, and the metabolic pool, the receptacle for discharged amino acid and the products of protein degradation. On the basis of the results of biochemical studies reported in the literature we propose that the amino acid is activated at the cell membrane. Therefore, only amino acid derived from the extracellular pool feeds the precursor pool. This compartmentalization would preclude mixing of the leucine derived from protein degradation with the precursor amino acid pool for protein synthesis. In vivo, however, because the extracellular space is small, this mixing might occur outside the cell.

We are currently carrying out experiments to test this model. One of our experiments consists of the determination of the specific activity of brain leucyl-tRNA and plasma leucine in a rat in a steady state for both labeled and unlabeled leucine in the plasma. If the leucine is reutilized, the specific activity of the leucyl-tRNA will never reach the specific activity of the plasma leucine because it will be constantly diluted by unlabeled leucine derived from protein degradation. We have worked out a schedule for the intravenous infusion of labeled leucine in order to achieve a constant plasma level. We have also developed a method for the extraction and determination of picomolar levels of leucyl-tRNA in brain. With the use of differential centrifugation, acid precipitation, and phenol extraction, yields of tRNA of 100-200 $\mu\text{g/g}$ brain can be achieved. Our best yields of leucine following deacylation of the tRNA at pH 10 are about 20 pmoles/g brain. Consequently we have had to develop an ultrasensitive method for determination of the level of leucine derived from

leucyl t-RNA. The method (adapted from a published method of Airhart et al., 1974) is based on the formation of labeled fluorescent amino acid derivatives following reaction of the amino acid extract with labeled dansyl chloride. The dansylated amino acids are separated by HPLC on a C-18 column, and the dansyl-leucine peak is collected and counted with double label liquid scintillation counting. Because of problems of a high number of counts in sample blanks due to dansyl chloride, dansylamide, and dansylic acid, a method was developed for separating the dansylated amino acids from these other derivatives. The techniques used include a preliminary step through a C-18 Sep Pak to remove unreacted dansyl chloride and ion exchange chromatography first with a cation exchange resin (AG-50) to remove dansylic acid followed by an anion exchange resin (AG-1) to remove dansylamide. Although losses of dansyl amino acids with this procedure are significant, blanks are reduced to background levels, and the accuracy of the determination of dansyl leucine is profoundly improved. With this method we can detect as little as 5 pmoles of leucine. The final results from this series of experiments will provide us with an answer to the question of reutilization of leucine derived from protein degradation and the half-life of the precursor pool. With these results we can test the validity of Model VI.

In another series of experiments we are determining the half-life of the precursor leucine pool in local brain regions. In these experiments animals are administered a programmed infusion of $L-[1-^{14}C]$ leucine designed to achieve and maintain a constant plasma level of $[^{14}C]$ leucine. We are determining the best-fitting rate constant for the turnover of the pool from the following measurements: the amount of label incorporated in protein following 5-15' infusions, the plasma leucine specific activity during the infusion, and the actual rate of synthesis as determined by pulse labeling experiments. Preliminary results indicate that the half-life of the pool in the frontal cortex in rat is 0.8 minutes.

In a third series of experiments we are determining the degree of dilution of the precursor pool by unlabeled leucine derived from protein degradation. In these experiments we are testing the effect of changing the plasma leucine concentration on the calculated rate of protein synthesis. If there is significant dilution of the precursor pool, the calculated rate of protein synthesis should vary directly with the plasma leucine concentration. The data obtained from this series of animals, i.e., the plasma level of leucine and $[^{14}C]$ leucine and the amount of $[^{14}C]$ leucine incorporated into protein, can be applied to a fitting routine in order to determine the best-fitting fractional dilution of the precursor pool.

The model that best fits the known biochemical behavior of leucine in brain is Model VI, a multicompartement model with a very complex operational equation. We have simplified this equation. We have established that we can easily remove all of the free leucine from tissue sections without affecting the concentration of $[^{14}C]$ protein in the tissue by formalin fixation and washing. The procedure that we have devised consists of immersion fixation of the tissue sections in 10% formalin (phosphate buffered, pH 7.0) followed by washing of the sections in six changes of formalin. This procedure simplifies our expression for the rate of protein synthesis such that the determination of the amount of label in each leucine pool is unnecessary. Thus the constants needed in the equation are: 1) the half-life of the precursor leucine pool and 2) the degree of dilution of

that pool. As our results suggest that the half-life is small (less than 1 minute), it can be shown that by 60 minutes after a pulse of [^{14}C]leucine the integrated plasma specific activity approximately equals the precursor pool specific activity. If the dilution factor is significantly different from 1, we are assuming that it does not change with our experimental conditions. Therefore, we are carrying out some studies on protein synthesis in brain in which we can obtain reasonable minimal estimates of the rates of protein synthesis. The other general assumptions of the method are:

- 1) steady state for protein and amino acid metabolism;
- 2) no breakdown of labeled protein;
- 3) tracer kinetics;
- 4) complete loss of $^{14}\text{CO}_2$ from the oxidation of L-[1- ^{14}C]leucine.

We have derived an equation that defines the rate of leucine incorporation into protein in terms of the following measurable variables: the time course of plasma-specific activity and the final tissue concentration of ^{14}C . The equation defines the procedure to be used and the variables to be measured. A pulse of [^{14}C]leucine (100 $\mu\text{Ci/kg}$ body weight) is administered intravenously, and the arterial plasma concentrations of labeled and unlabeled leucine are monitored for the duration of the experimental period. At 60 minutes the animal is killed by an intravenous injection of pentothal. The brain is removed, frozen, sectioned and washed and local tissue concentrations of ^{14}C are determined by quantitative autoradiography. We have calibrated new [^{14}C]methylmethacrylate standards for quantitative autoradiography with 10, 20, and 30 μm sections of brain. These standards are in a lower range than those used in the deoxyglucose method and will be more suitable for leucine autoradiographs.

We have determined rates of protein synthesis in a number of brain regions. We find a wide range of values from 1.5 nmoles leucine/g of tissue/min in white matter to 20 nmoles/g/min in some hypothalamic nuclei (e.g., the supraoptic nuclei). In general, brain regions that are rich in nerve cell bodies, such as the pyramidal cell layer in the hippocampus, and cranial nerve nuclei such as the dorsal motor nucleus of the vagus, have high rates of protein synthesis as compared to either white matter or regions composed largely of nerve terminals, dendrites, synapses, and axons, such as the caudate nucleus, thalamus and cortex. The value that we have obtained for cortex (5.0 nmoles leucine/g/min) compares favorably with values obtained by Dunlop et al. (1975) of 4.7 nmoles valine/g/min with a completely different method that yields only average values for the brain as a whole.

We are also carrying out several studies on the effects of specific treatments or conditions on local rates of protein synthesis. The purpose of some of these studies is to test the sensitivity of the method to detect changes in local rates of protein synthesis as well as to determine the responsiveness of protein synthesis to altered physiological states or pathological conditions. Experiments done in collaboration with Dr. R. Collins have shown that chemically-induced focal seizures produce a reduction in protein synthesis while stimulating glucose utilization. Studies on the effect of injury to the hypoglossal nerve have been carried out in collaboration with Dr. B. Agranoff. These studies have shown that cutting the hypoglossal nerve on one side will result in an increase in protein synthesis in its nucleus. We have studied the

time course of this effect. We have also examined the time course of the effect of nerve section on glucose utilization in the nucleus. Our results show that both of these processes increase in the nucleus ipsilateral to the sectioned nerve and are unaffected in the contralateral nucleus as compared with sham-operated animals. The time courses of these metabolic changes have been compared with that of the return of functional innervation of the tongue. An increase in glucose utilization is first detected 24 h post-axotomy. It is maximal between 1 and 3 days post-axotomy and constitutes an 84% increase over the rate in the contralateral control nucleus. The increase in protein synthesis is of smaller magnitude than that of glucose utilization. It is maximal at 48 h following axotomy and constitutes a 25% increase over the rate in the contralateral nucleus. The increases in both of these metabolic processes persist even after functional recovery of the tongue at 21 days post-axotomy. Protein synthesis and glucose utilization return to normal levels between 24 and 35 days post-axotomy. Although the time courses of the changes in protein synthesis and glucose utilization are similar, the magnitude of the increase in glucose utilization is too large to be accounted for by the energy requirements of the relatively small increase in protein synthesis and probably reflects other processes as well, including altered function of the soma-dendritic membrane of regenerating neurons. A manuscript of these results is in press.

A study of the effects of chronic hypothyroidism and cerebral protein synthesis in adult rats was carried out. Two groups of male rats were studied: 1) rats which were surgically thyroidectomized three months prior to the study and 2) sham-operated controls. Of the 51 brain regions examined there were significant decreases in the rates of protein synthesis in 13 structures of the hypothyroid animals. These structures included mainly components of the extrapyramidal motor system, nuclei of cranial nerves, and hypothalamic nuclei. There were no significant changes in visual or auditory pathways or in any region of the cortex. Chronic hypothyroidism, therefore, appears to decrease rates of protein synthesis in a few selected areas of brain. These results are of interest because altered thyroid function in adults has been shown to influence the activity of several brain enzymes, to alter neurotransmitter levels and to modify behavior. A manuscript of these results is in preparation.

Experiments on the effects of pentobarbital anesthesia on local rates of cerebral protein synthesis in adult rats have been carried out. Barbiturate anesthesia has a profound effect on cerebral glucose utilization, and it was of interest therefore to examine its effects on another biochemical measure such as protein synthesis. Two series of animals were studied: normal conscious rats and barbiturate anesthetized animals. The results of these experiments are being analyzed.

In collaboration with the Unit on Sleep Studies, and the Laboratory of Neuropsychology, we are also studying the effects of slow wave sleep on local rates of protein synthesis in monkey. This study is designed to test a long held hypothesis that sleep is a physiologic period during which the brain tissue undertakes repair and remodeling. To date 3 awake and 3 asleep monkeys have been studied. Local rates of protein synthesis have been determined in 80 brain regions with the use of the computer-assisted image-processing system. These preliminary results show a general increased rate of cerebral protein synthesis

throughout the brain. Statistically significant increases occur in 7 of the brain regions examined including the pulvinar, lateral habenula, interpeduncular nucleus, magnocellular reticular nucleus, dorsal motor nucleus of the vagus, claustrum and accessory auditory cortex.

Significance to Biomedical Research and Program of the Institute.

Protein synthesis is probably the most important biochemical process underlying the development, maturation, plasticity, maintenance, and long-term regulation of the nature and degree of functional activity of the nervous system. The structural, functional, and metabolic properties of the tissues largely reflect the role of structural and enzymatic proteins. Peptides that are considered to be neurotransmitters are in some, and possibly all, cases derived from the cleavage of large parent protein molecules. Many hormones within and outside the nervous system are proteins. It is, therefore, certain that changes in protein synthesis can and do alter function and that some mental and neurological dysfunctions reflect disturbances in this vital biochemical process. This research is directed at determining the rates of protein synthesis in specific regions of the central nervous system with an ultimate resolution down to the cellular level. This provides for the first time the opportunity to study at the individual structural or anatomical level the changes in protein synthesis that may be the causes, consequences, or correlates of normal conditions, such as maturation, plasticity, differentiation, sleep, learning and memory, behavioral patterns, etc., or pathological conditions, such as hormonal disorders, aging, regeneration in response to injury, convulsive disorders, coma, etc.

Proposed Course.

We are continuing our efforts on completing experiments on the question of dilution of the precursor pool specific activity with leucine derived from protein degradation. In the same experiments we are determining again the half-life of the precursor pool. The applications of the method to the study of slow wave sleep will be continued.

Publications:

Sokoloff, L., and Smith, C.B.: Basic principles underlying radioisotopic methods for assay of biochemical processes in vivo. In Lambrecht, R.M. and Rescigno, A. (Eds.): Tracer Kinetics and Physiologic Modeling. Berlin/Heidelberg/New York/Tokyo. Springer-Verlag, 1983, pp. 202-234.

Sokoloff, L., and Smith, C.: Biochemical principles for measurement of metabolic rates in vivo. In Heiss, W.-D. and Phelps, M.E. (Eds.): Positron Emission Tomography of the Brain. Berlin/Heidelberg/New York, Springer-Verlag, 1983, pp. 2-18.

Sokoloff, L., and Smith, C.B.: Basic principles underlying radioisotopic methods for assay of biochemical processes in vivo. In The Metabolism of the Human Brain Studied with PET, (Nobel Conference VII, May 17-20, 1983). Raven Press Books, Ltd., London (in press) 1983.

Smith, C.B.: The influence of age on cerebral energy metabolism. In Energy Transduction and Neurotransmission, Joint ESN-WFN Symposium, Rome, September 20-21, 1982. (In press) 1984.

Ingvar, M.C., Maeder, P., Sokoloff, L., and Smith, C.B.: Effects of ageing on local rates of cerebral protein synthesis. Brain (in press) 1984.

Smith, C.B., Crane, A.M., Kadekaro, M., Agranoff, B., and Sokoloff, L.: Stimulation of protein synthesis and glucose utilization in the hypoglossal nucleus induced by axotomy. J. Neurosci. (in press) 1984.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02216-01 LCM

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Metabolic Mapping of the Brain during Rewarding Self-Stimulation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

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COOPERATING UNITS (if any)

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LAB/BRANCH

Laboratory of Cerebral Metabolism

SECTION

Developmental Neurochemistry Section

INSTITUTE AND LOCATION

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TOTAL MAN-YEARS:

2

PROFESSIONAL:

1.5

OTHER:

.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The deoxyglucose method is being used to study alterations in local cerebral glucose utilization in rats during the performance of goal-oriented self-stimulation behavior. By mapping metabolic activity in rats during electrical stimulation to various discrete areas of the brain, information can be obtained about those areas of the brain involved in motivation, learning, and reinforcement of behavior. Further, by using the deoxyglucose method to assess the effects of drugs such as the psychostimulants and opiates on rewarding electrical stimulation, the specific regions or systems in the brain which mediate the reinforcing properties of these drugs can be identified.

Project Description:

The 2-[¹⁴C]deoxyglucose method affords a novel and unique opportunity to map functional neural pathways simultaneously in all anatomical components of the central nervous system. This method, therefore, allows the identification of complex neural circuits that are functionally active during behavioral or pharmacological manipulation.

The goal of this project is to map regions of the rat brain activated during rewarding electrical brain self-stimulation. The self-stimulation phenomenon in which rats perform an operant task in order to receive brief trains of electrical pulses directly to various regions of their brains is recognized as a model of goal-oriented behavior and as a way to study the neural basis of such behavior.

Our previous work in which local rates of glucose utilization were measured in rats lever pressing to receive electrical stimulation to the ventral tegmental area (VTA) of the midbrain revealed a selective pattern of metabolic activation in the terminal fields of the VTA including the prefrontal cortex, nucleus accumbens, amygdala, lateral septum, and locus coeruleus. We also found increases in sensory and motor structures involved in the performance of the lever-pressing task. These results show that self-stimulation to the VTA is associated with discrete activation of specific neuronal projection fibers and selective terminal sites.

The ventral tegmental area which contains dopaminergic cell bodies was chosen as the site of self-stimulation because of the evidence for dopaminergic mediation of this behavior. In a followup study we used the quantitative 2-[¹⁴C]deoxyglucose autoradiographic method to examine self-stimulation to the substantia nigra pars compacta (SNc), an area that also contains dopaminergic cell bodies but has anatomically different afferent and efferent connections.

This convergence of metabolic activation despite differences of anatomical innervation suggests a significant role for these regions in mediation of goal-oriented or motivated behavior. The results of this study confirm that the performance of goal-oriented self-stimulation behavior is associated with widespread bilateral changes in metabolic activity throughout the brain. Specifically, increases in glucose utilization were found in terminal areas of the SNc including the caudate and anterior cingulate. In addition, changes were found in areas which receive few direct projections from the SNc, in particular the medial prefrontal cortex and nucleus accumbens. These changes were similar to those seen in the study of self-stimulation to the VTA.

In a previous experiment, we showed that the pattern of changes in LCGU found in self-stimulating animals is specific to this behavior and not merely the result of the electrical stimulation itself. When glucose utilization in self-stimulating animals was compared to that in animals which received noncontingent electrical stimulation delivered by the experimenter (at rates and current parameters comparable to those of the self-stimulating rats) to ventral tegmental area placements shown to support self-stimulation a very different pattern of changes was found.

We have extended these findings demonstrating that the effects of experimenter-administered stimulation to the substantia nigra pars compacta are different from self-stimulation to the nigra. The pattern of LCGU changes were clearly distinguishable in the self-stimulation and experimenter-administered stimulation groups. Particularly noteworthy were bilateral increases in LCGU in the medial prefrontal cortex, caudate nucleus, nucleus accumbens and the ventral pallidus in the ICSS group, which were not activated in the EAS group. These data indicate that the distribution of changes in LCGU found in ICSS rats is the result of the goal-oriented nature of their behavior and not simply the consequence of electrical stimulation.

The differences seen in these experiments serve to emphasize the importance of the method of presentation of the brain stimulation and the context in which it is presented. In order to examine this issue further we studied this question behaviorally. We showed that rats who had previously self-stimulated at high rates would work to escape or turn off brain stimulation to the ventral tegmental area when it was presented by the experimenter even at rates and current intensities comparable to those which supported self-stimulation. Although brain stimulation to the VTA is a positive reinforcer in that rats will work to receive such stimulation, brain stimulation to the VTA can also be a negative reinforcer as in this case in that rats will perform an operant response to terminate its non-contingent presentation. This suggests that the aversiveness of such stimulation is due to the lack of self-regulation by the subject rather than any intrinsic properties of the underlying tissue.

It is well-known that amphetamine pretreatment induces an increase in the rate of responding for self-stimulation. In another set of experiments we used the 2-deoxyglucose method to study the effects of this drug in the substrates of self-stimulation to the ventral tegmental area. The results of these experiments in which amphetamine (0.5 mg/kg) was administered to animals lever-pressing for stimulation to the VTA at current levels one-third those used in our earlier work revealed a complex pattern of alterations in metabolic activity which was similar in many areas to those changes seen in animals receiving either drug or stimulation alone. In other areas, however, effects were seen which were unique to this combination of treatments, indicating that the facilitative actions of amphetamine on rewarding brain stimulation may be exerted in areas that are not predicted by the effects of rewarding brain stimulation alone or amphetamine's actions on other behaviors.

Significance to Biomedical Research and Program of Institute.

The 2-deoxyglucose method has allowed for the first time the simultaneous visualization of the functional neural circuits specific to the performance of self-stimulation behavior. Because self-stimulation is a basic model of appetitive goal-oriented behavior, elucidating the areas of the brain involved in this behavior can provide information about the neural systems involved in motivated and learned behavior.

Further, because self-stimulation behavior is facilitated by both opiates and psychostimulants, the two most widely abused and euphorogenic classes of

drugs, the study of the effects of these agents on self-stimulation should lead to important insights concerning the precise site and mechanisms of the reinforcing actions of these drugs.

Proposed Course.

The application of the 2-deoxyglucose method to studies of rewarding brain stimulation will be extended to include other self-stimulation sites in the brain, particularly sites within the dorsal noradrenergic bundle. This will give us information about whether common or independent systems are involved in reward processing. Second, our behavioral studies of escape from noncontingently applied brain stimulation will be continued by assessing this behavior at other brain sites in order to test the hypothesis that the magnitude of reward is inversely related to the stress or aversiveness of noncontingently applied brain stimulation. Finally, metabolic studies of self-stimulation to the ventral tegmental area will continue in order to relate the pattern of metabolic activation to the level of rewarding brain stimulation. This parametric study will provide information about which areas are essential for the expression of goal-oriented self-stimulation behavior and provide the basis for the further study of the changes that result in goal-oriented behavior following the administration of psychostimulants and opiates.

Publications:

Porrino, L.J., Esposito, R.U., Seeger, T.F., Crane, A.M., Pert, A., and Sokoloff, L.: Metabolic mapping of brain during rewarding self-stimulation. Science 224: 306-309, 1984.

Esposito, R.U., Porrino, L.J., Seeger, T.F., Crane, A.M., Everist, H.D., and Pert, A.: Changes in local cerebral glucose utilization during rewarding brain stimulation. Proc. Natl. Acad. Sci. 81: 635-639, 1984.

Porrino, L.J., Lucignani, G., Dow-Edwards, D., and Sokoloff, L.: Dose-dependent effects of acute amphetamine administration on functional brain metabolism in rats. Brain Res. 307: 311-320, 1984.

NOTICE OF INTRAMURAL RESEARCH PROJECT

201 MH 02217-01 LCM

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Plasticity in the Developing Monkey Visual System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I. Carolyn B. Smith Senior Staff Fellow LCM, NIMH

Others: Louis Sokoloff Chief, Lab. Cerebral Metabolism LCM, NIMH

COOPERATING UNITS (if any)

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LAB/BRANCH

Laboratory of Cerebral Metabolism

SECTION

Developmental Neurochemistry Section

INSTITUTE AND LOCATION

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TOTAL MAN-YEARS

.60

PROFESSIONAL

.30

OTHER

.30

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type Do not exceed the space provided)

The postnatal development of the central visual pathways depends on the quality of the visual environment. During the critical period in the primate visual system environmental manipulation can modify the physiological properties of visual cortical cells. The purpose of this project is to study the underlying biochemical events that imbue the nervous system with the property of plasticity. Protein synthesis is a biochemical process which is involved in bringing about changes in morphology, adjustments in growth rates, and remodeling and maintenance of structures. We have therefore used the [¹⁴C]leucine method to study the relationships between local plastic changes which occur in the developing monkey visual system and local rates of protein synthesis.

Project Description:

The postnatal development of the central visual pathways depends on the quality of the visual environment. During the critical period environmental manipulation can modify the physiological properties of visual cortical cells. If a monkey is monocularly deprived during the first few weeks of life there is a reorganization of the striate cortex such that the ocular dominance columns representing the functioning eye extend their boundaries and broaden at the expense of the adjacent columns representing the deprived eye. Eventually, most of the striate cortex may be incorporated into a monocular visual system that serves only the deprived eye. The organization of the lateral geniculates (dLGN), the locus of the cell bodies of the terminals in striate cortex, remains normal.

The purpose of this project is to study biochemical events underlying these plastic changes. We have focused initially on the process of protein synthesis because it is a requirement for growth and development and because changes in morphology and rates of growth and remodeling and even maintenance of existing structures should be reflected in changes in rates of protein synthesis. These relationships may hold true only if rates of protein synthesis can be determined on the intact system and with enough regional specificity to permit examination of the region of interest. With the advent of the [^{14}C]leucine method for the determination of local rates of protein synthesis in brain *in vivo*, such a study is now possible. The [^{14}C]leucine method was therefore used to first define any changes in protein synthesis that take place in conjunction with plastic changes in the developing monkey visual system and then to examine the source of regulation of any changes that occur.

In early studies the effects of acute and chronic monocular deprivation in the newborn rhesus monkey on rates of protein synthesis in the cell bodies of origin of the terminals in layer IV of the striate cortex were examined. These cell bodies are located in well-defined layers segregated for the right and left eyes in the dLGN. The results showed that in newborn monkeys with chronic monocular deprivation as compared with normal monkeys with binocular vision there were decreases of about 15% in the rates of protein synthesis in the laminae innervated by the deprived eye whereas in geniculate laminae innervated by the functioning eye rates of protein synthesis were normal. Acute monocular deprivation produced no differential changes in rates of protein synthesis in any of the dLGN laminae. These results suggest that the underdevelopment of the deprived columns in striate cortex is the result of inadequate growth and/or maintenance of axon terminals with consequent default of synaptic connections to the normally maintained terminals of the functional pathway.

Results of neurophysiological and anatomical experiments carried out in other laboratories suggest that it is the competition for synapses in the striate cortex that is affected by monocular deprivation and which ultimately leads to the domination of the cortex by the input from the non-deprived eye. Our protein synthesis results are consistent with this hypothesis and they suggest that changes in rates of protein synthesis may be involved in this reorganization. Alternatively, the effects on protein synthesis may be a consequence of this reorganization rather than an influence on it. In other

words, protein synthesis in the cells of the dLGN may be regulated by the organization of the terminals in layer IV of the striate cortex or it may be regulated by the input or lack of input from the retina. If it is regulated by input from the retina, then protein synthesis in the cells of the dLGN may be in turn regulating the reorganization of the terminals in striate cortex.

In order to distinguish between these two possibilities we have studied the effects of binocular deprivation and recovery from monocular deprivation on protein synthesis. Physiological experiments have shown that binocular deprivation during the critical period results in a normally organized striate cortex where monocular deprivation followed by binocular vision results in a striate cortex still dominated by the originally nondeprived eye. In one monkey which was allowed 25 days of binocular vision following 25 days of monocular deprivation, rates of protein synthesis in the previously deprived eye layers of the LGN were reduced by 20% compared to values in age-matched controls, whereas rates of protein synthesis in the nondeprived eye layers were normal. This result is consistent with the hypothesis that the regulation of protein synthesis in the dLGN is occurring retrogradely; i.e., from the terminals in the striate cortex. In experiments on the effects of binocular deprivation during the first 25 days of life rates of protein synthesis were significantly reduced by about 20% in all of the laminae of the dLGN. Although it is known from physiology experiments that the competitive advantage is not upset by binocular deprivation, this procedure may have influenced the extent of all of the terminal fields in the striate cortex.

In another set of experiments monkeys monocularly deprived during the first 25 days of life were reverse-sutured; i.e., on day 25 the deprived eye is opened and the nondeprived eye was sutured and remained sutured for an additional 25 days. In 2 reverse-sutured monkeys, rates of protein synthesis in the newly deprived layers were reduced by about 15% as compared with age-matched controls, whereas protein synthesis in the previously deprived layers was normal. In the reverse-sutured monkeys, in contrast to all of the other monkeys studied, changes in protein synthesis in the striate cortex can be visualized on the [^{14}C]leucine autoradiographs. In reverse-sutured animals columns with alternating high and low rates of protein synthesis were evident. The columns were perpendicular to the cortical surface with a periodicity of about 0.8 mm. They were particularly distinct in layers 2-4. The portion of the period with the higher rate of protein synthesis was slightly wider. These results show that in the newborn monkey the reorganization that occurs in response to chronic visual deprivation is reflected in changes in protein synthesis in the cells along the visual pathway. The reduction in protein synthesis in the dLGN may underlie an inadequate maintenance of terminals in the striate cortex with a consequent loss of the competition to afferents from the nondeprived eye. The results of this reverse suture experiment suggest that the reorganization of the visual cortex that takes place in response to this experimental procedure also involves changes in protein synthesis in cortical cells.

Other experiments in progress are designed to further examine the processes involved in the plasticity demonstrated by this system. In addition, we have studied several prepubescent monkeys to test whether or not this protein synthesis response occurs in monkeys with fully developed visual systems. In

two monkeys: 1) one year old monocularly deprived for 3 months, and 2) 18 month old monocularly deprived for 6 months, we found no significant effect on the rates of protein synthesis in the laminae of the lateral geniculates. We know, however, that in these monkeys that some reorganization at the level of the striate cortex has taken place because in another 18 month old monkey, monocularly deprived for 6 months, our deoxyglucose autoradiograph showed some irregularities in the metabolic picture of the ocular dominance columns.

Significance to Biomedical Research and Program of the Institute.

Plasticity, the capacity of the nervous system to respond to changes in the environment, is one of the most fundamental properties of nervous tissue. Learning, a form of plasticity, is a process of intense interest to neuroscientists the world over. In an attempt to study some of the biochemical processes underlying plastic changes, we have embarked on this study of the developing monkey visual system, about which the physiology and anatomy are well-known. Studies with the [^{14}C]leucine method for local rates of protein synthesis and the [^{14}C]deoxyglucose method for local rates of glucose utilization are directed at first a description of some of the biochemical events which occur and then a determination of the regulation of these events. The understanding of these events may provide some insight into the unique properties of the critical period which make it so responsive to environmental manipulation. In addition, this research may have some direct implications on the clinical management of children with congenital cataracts and strabismic amblyopia.

Proposed Course.

As the present studies are quantitative in nature, it is necessary to have 4 to 5 animals in each group in order to use statistical tests for significant differences. We are therefore repeating a number of the studies in order to apply these tests. In experiments already completed, we are analyzing structures further along the visual pathway. Future plans include additional manipulations of visual input particularly during the latter portion of the critical period.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02220-01 LCM

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Regional Biochemical Changes in the Normal Aging Brain

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I. Carolyn B. Smith Senior Staff Fellow LCM, NIMH

Others: Louis Sokoloff Chief, Lab. Cerebral Metabolism LCM, NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Cerebral Metabolism

SECTION

Developmental Neurochemistry Section

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

.60

PROFESSIONAL:

.30

OTHER:

.30

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies are being carried out on the effects of aging on cerebral protein synthesis and glucose utilization in rats. With the application of local methods developed in this laboratory discrete regions of the brain can be examined in normal conscious animals. The regional changes in glucose utilization indicate that entire sensory pathways are affected by the aging process. The fact that similar changes are found in the same pathways with respect to protein synthesis suggests that some of these changes reflect an adaptation of the nervous system to a chronic lack of input. The basis of some of the changes which occur with age can be further examined in studies with pharmacological agents as well as in conjunction with behavioral measurements.

Project Description:

Aging is generally associated with a reduction in cerebral functional capacity. Elderly subjects, even those highly selected for freedom from disease, exhibit general slowing of reaction times and reduced levels of performance on tests of psychomotor functions and nonverbal intelligence. Blood flow and oxygen consumption of the brain as a whole remain normal in elderly subjects while glucose consumption is reduced. Anatomical studies have revealed a number of degenerative changes in senescent brains; these changes have a predilection for certain brain regions. In view of the specificity of some of the senescent changes in human subjects the question of regional biochemical changes was examined in these studies.

The present studies have been carried out on normal albino rats. Normal aging in the rat is also accompanied by some behavioral as well as histopathological changes in the brain. The use of the rat in such studies is advantageous in that it is relatively free of arteriosclerosis which eliminates confounding secondary effects of vascular pathology. The autoradiographic deoxyglucose method was applied to examine the effects of normal aging on local cerebral glucose utilization. Decreases with age were found in the brain as a whole. On a local level senescent decreases were found with the most profound effects in the components of the primary auditory and visual pathways. These were effects similar to those seen following sensory deprivation of these systems. These results raised the question of whether or not some of the central nervous consequences of normal aging might not be due to sensory deprivation due to sense-organ degenerative changes inasmuch as there is known to be some retinal and inner ear degenerative change with age. With only a few exceptions the rates of glucose utilization in structures of the limbic and motor systems remained unchanged with age. The exceptions were several regions of white matter and the caudate-putamen.

The caudate-putamen which is part of the nigrostriatal dopaminergic system is a prominent site of changes during senescence. Signs of Parkinsonism are common in normal aged human subjects and movement disorders which can be reversed by dopamine agonists have been observed in aged rats. Changes with age in dopamine turnover, the number of dopamine receptors and the dopamine sensitivity of adenylate cyclase in the striatum are well documented. In order to further examine the functional consequences of these reported changes in the nigrostriatal system the deoxyglucose method was used to study the effects of normal aging on the metabolic responsiveness of dopamine-receptor activation by systemically-administered apomorphine. Significant dose-dependent effects of this dopamine-agonistic drug were found in 6 of the 14 brain regions examined in the young rats. In the lateral habenula and anterior cingulate cortex the effect of apomorphine was to decrease the rate of glucose utilization whereas in the subthalamic nucleus, inferior olivary nucleus, substantia nigra (pars compacta), and substantia nigra (pars reticulata) apomorphine stimulated glucose utilization. Age-dependent changes in responsiveness to apomorphine were found in the subthalamic nucleus, substantia nigra (pars reticulata), and inferior olivary nucleus. In the subthalamic nucleus the stimulation of glucose utilization by apomorphine was decreased in the old rats at all doses including those that elicited maximal responses. In the inferior olivary nucleus and the

substantia nigra (pars reticulata) the dose-response curves were markedly depressed in the aged group. No significant effect of apomorphine on the rate of glucose utilization was found in the caudate-putamen as a whole, but a significant stimulation of glucose utilization was found only in the ventral portion in the young animals. The significant age-dependent decreases in responsiveness to apomorphine found in the subthalamic nucleus, substantia nigra (reticulata) and inferior olivary nucleus may reflect the functional consequences of the reported loss of dopamine receptors in the caudate-putamen with aging.

Measurements of energy metabolism do not differentiate between the immediate functional demands of cerebral structures and the longer term maintenance processes within the nervous system. Long term effects that are related to changes in morphology, structural maintenance, and remodeling in the nervous system are more likely reflected in biosynthetic biochemical processes, such as protein synthesis. In another study the effects of aging on local rates of protein synthesis in brain were examined by means of the quantitative autoradiographic [14 C]leucine method. The results show that aging is associated with significant decreases in rates of protein synthesis in the brain as a whole as well as in several specific brain regions. Brain regions involved in visual and auditory function were selectively affected, perhaps due to a chronic lack of sensory input. Several regions involved in motor function and 2 areas in the limbic system had significantly decreased rates of protein synthesis in the old rats. Notably, there was a significant age-related decrease in protein synthesis in the locus coeruleus which contains the cell bodies of origin of the major ascending noradrenergic innervation of the cortex.

Significance to Biomedical Research and Program of the Institute.

Insofar as aging is rapidly becoming a problem of increasing social significance this research which is focused on senescent changes in the ability of the brain to function may be of considerable importance to the medical community. Furthermore, our results indicate that some of the changes that occur with age may be the consequence of a decreased functional activity. Confirmation of this possibility and further understanding of the basic biochemical processes underlying plastic changes in the nervous system of either an involuntal or developmental nature may be useful in trying to prevent and/or reverse such senescent changes.

Proposed Course.

Completed experiments on the effects of aging on protein synthesis and glucose utilization will be further analyzed. In particular, the nucleus basalis in which there are known pathological changes in Alzheimer's Disease will be examined. In addition a collaborative effort with Dr. M. Diamond at the University of California (Berkeley) is being set up to study the effects of environmental enrichment of cerebral metabolism in aged rats.

Publications:

Ingvar, M., Maeder, P., Sokoloff, L., and Smith, C.B.: The effects of aging on local rates of protein synthesis in rat brain. In Fieschi, C., Lenzi, G.L., and Loeb, C.W. (Eds.): Effects of Aging on Regulation of Cerebral Blood Flow and Metabolism. Basel, Karger, 1984, pp. 47-50.

Smith, C.B.: Aging and changes in cerebral energy metabolism. Trends Neurosci. 7: 203-208, 1984.

Smith, C.B.: A comparison of the effects of aging on local rates of cerebral glucose utilization and protein synthesis in Sprague-Dawley rats. In Proceedings of SIR International Symposium on The Need for Treatment for Stroke, Alzheimer's Disease and Abnormal Brain Aging (New York, September 20-22, 1984). London, England, John Libbey Eurotext Limited, (in press) 1984.

Smith, C.B.: Effects of aging on local rates of cerebral energy metabolism and protein synthesis in Sprague Dawley rats. In Depresseux, J.C. (Ed.): Cerebral Circulation and Metabolism. Montrouge, France, John Libbey Eurotext Limited, (in press) 1984.

Sokoloff, L.: The aging brain: Introduction. In Depresseux, J.C. (Ed.): Cerebral Circulation and Metabolism. Montrouge, France, John Libbey Eurotext Limited, (in press) 1984.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00900-28 LCM

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biochemical Studies on Myelin and Myelin Basic Protein

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

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Others: G. E. Deibler Chemist LCM, NIMH
M. L. Pedersen Biologist LCM, NIMH
A. Stone Research Chemist LNB, NIMH

COOPERATING UNITS (if any) Lab. Experimental Carcinogenesis, NCI, NIH (Brown, L.)
Neuropathology Dept., Univ. of Washington Sch. of Med., Seattle, Wash. (Alvord,
E.C., Jr.); Sch. of Chemistry, Univ. of Sydney, New South Wales, Australia
(Hoore, W.); Michigan Molecular Inst., Miedland, Mich. (Mendz, G.);

LAB/BRANCH

Laboratory of Cerebral Metabolism

SECTION

Section on Myelin Chemistry

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

3

PROFESSIONAL:

1.5

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We are continuing to pursue the following avenues of work: 1) nuclear magnetic resonance studies to help elucidate the detailed conformation of the myelin basic protein and 2) immunochemical studies aimed at delineating antigenic sites and at exploring the tertiary structure of the protein. In addition, we are 3) examining large fragments of the protein with regard to their potential for forming α -helical secondary structure, 4) defining the amino acid sequence of the second site in the protein responsible for the induction of autoimmune encephalomyelitis in rabbits, and 5) determining the sites of cleavage of the protein by plasmin.

Project Description:Objectives:

The major objective of the current work has been to elucidate the three-dimensional structure of the myelin basic protein (BP) in aqueous solution and when bound to phospholipids or comparable amphipathic molecules in an attempt to understand the function of the protein in the myelin sheath.

Methods Employed:

The work has involved nuclear magnetic resonance (NMR) and immunological studies of the BP and specific fragments thereof from a number of species as well as the preparation of large quantities of the BP fragments useful in such studies.

Major Findings:

1. We have recently determined the complete amino acid sequence of pig BP. The sequence is very similar to that of bovine BP except for several conservative substitutions, the most interesting of which are the Ser→Pro substitutions, which occur at positions 133 and 137. These Pro residues must cause obligatory abrupt foldings of the polypeptide chain in the sequence Ala-Pro-Asp-Tyr-Lys-Pro-Ala-His (132-139). Since Ser residues can readily substitute for Pro residues in such folded conformations (reverse turns), one might expect on theoretical grounds that in the BP of both species, the conformation of sequence 132-139 would be two sequential reverse turns, Ala-X-Asp-Tyr and Lys-X-Ala-His, where X is either Ser or Pro. Direct evidence for the second turn has been provided by NMR studies, which indicate a close proximity of Tyr¹³⁵ and His¹³⁹ in the bovine BP sequence Tyr-Lys-Ser-Ala-His (135-139).

2. Studies carried out in collaboration with Drs. Walter Moore, George Mendz, and Larry Brown have demonstrated that binding of the BP to dodecylphosphocholine micelles (a model for a lipid membrane) induces α -helix formation in several fragments of the protein, which are otherwise relatively devoid of linear repeating secondary structure. Since the helical content (~ 28 residues) of peptide 1-98 bound to the micelles is approximately twice that of peptide 1-90, a major helical segment must be contributed by sequence 91-98. We predict that it involves sequence Pro-Val-Val-His-Phe-Phe-Lys-Asn-Ile-Val-Thr (86-96). The remaining helical residues in peptide 1-98 cannot be accounted for by helices within peptides 1-44 and 45-90, since the latter peptides do not become helical when bound to the micelles. The cleavage of peptide 1-90 (~ 13 helical residues) at the Phe⁴⁴-Phe⁴⁵ bond would appear to destroy a helical region. We predict that this helical region includes sequence Ile-Leu-Asp-Ser-Leu-Gly-Arg-Phe-Phe (37-45). The helical sequence (~ 11 residues) in peptide 99-172 has likewise not been precisely located. On the basis of secondary structure predictions, we expect helix formation to occur in sequence Thr-Leu-Ser-Lys-Ile-Phe-Lys-Leu (151-158). All three of the sequences mentioned are capable of forming strongly amphipathic helices, with nonpolar and polar faces. In the presence of lipids or detergents the α -helical conformation would be stabilized, since in this conformation the nonpolar amino acid side chains would be removed from solvent by partial penetration into the hydrocarbon phase of the micelle.

NMR studies carried out on the intact BP have shown, by chemical shift changes of the resonances of specific Met, Thr, His, and Tyr residues (and by line broadening of these and other resonances by fatty acid spin labels incorporated into the micelles), some of the residues which are involved in the micelle binding. These include a number of residues from His¹⁰ to His³², from Val⁸⁷ to His⁸⁹, and from Trp¹¹⁷ to Tyr¹³⁵. Residues from His⁶¹ to His⁷⁸ and residues Arg¹⁰⁸, His¹³⁹, and Met¹⁶⁹ do not interact with the lipid. Since the resonances of individual Leu, Ile, and Phe residues have not been defined precisely, interaction of predicted helices 37-45 and 151-158 with the lipid micelles cannot be unequivocally demonstrated at the present time. We do know however, that residues from His¹⁰ to His³² are not in α -helical conformation when bound to the micelles, whereas Val⁸⁷ and His⁸⁹ are.

In a related project, carried out in collaboration with Dr. Audrey Stone, various fragments of the BP are being examined for their tendency to become helical in solutions of trifluoroethanol, a solvent that stabilizes this conformation. So far, the studies have shown that different fragments differ with regard to both the concentration of trifluoroethanol required to promote helix formation and the maximum length of helix induced at high concentrations of the solvent.

3. In a collaborative study with Dr. E. C. Alvord, Jr., we have been continuing our studies on antigenic sites (epitopes) of the BP recognized by monoclonal antibodies (MAbs). We have found that one of the MAbs cross-reacts with two different epitopes. One of these consists of sequence Ser-Arg-Phe-Ser-Trp-Gly-Ala-Glu (113-120), whereas the other is located within sequence 39-73 of the BPs of certain species (guinea pig and bovine) and includes sequence Phe-Phe (44-45). This MAb is distinct from two others that are truly monospecific but whose complete epitope appears to consist of regions widely separated in the amino acid sequence. One MAb is directed at a sequence that includes Phe-Phe (90-91) plus as yet undefined residues in sequence 1-44. The other MAb is directed against an epitope in guinea pig BP that includes a sequence around Tyr¹³⁵ plus as yet undefined residues in sequence 1-90. We are currently testing peptides 22-169 and 45-172 from guinea pig BP to determine how much of the BP is required for full reactivity toward the MAbs. These studies are of particular importance in that they should be able to reveal how the polypeptide chain folds back on itself to form a three-dimensional structure.

4. A second region of the BP capable of inducing experimental autoimmune encephalomyelitis in rabbits is under investigation. Studies with peptic, thrombic, and tryptic fragments of the BP have shown that, in addition to sequence Thr-Thr-His-Tyr-Gly-Ser-Leu-Pro-Gln-Lys (66-75), a second encephalitogenic site is present in peptides 1-31 and 15-44. Activity appears to require the presence of Met²¹, since oxidation of this residue to methionine sulfoxide with H₂O₂ destroys the activity of peptide 15-44. Interestingly, peptide 1-21 in which Met²¹ has been converted to homoserine lactone (by BrCN cleavage of the Met²¹-Asp²² bond) is weakly active, whereas peptide 14-25 is inactive. We are currently investigating the effect of removal of Leu¹⁵ and Ala¹⁶ on the activity of peptide 15-44, the effect of regenerating methionine by reduction of the methionine sulfoxide, and the effect of reversible methylation of Met²¹.

5. Stimulated macrophages secrete plasminogen activator, which converts circulating plasminogen to plasmin. This process, which may occur in inflammatory demyelinating diseases, has been shown, in vitro, to result in the degradation of myelin basic protein in isolated myelin. In order to determine the sites in the basic protein cleaved by plasmin, as well as to obtain peptides for possible physicochemical and immunologic studies, we have subjected rabbit basic protein to partial plasmic cleavage. Under the conditions used essentially all of the basic protein was cleaved, but many large fragments remained. Peptides were isolated from the digest by ion-exchange chromatography on carboxymethyl-cellulose at pH 8.2 and at pH 4.7 and by gel filtration through Sephadex G-75 and G-50 in 0.01 M HCl and identified by tryptic peptide mapping and amino acid analysis. The results showed that plasmin cleaves primarily at Lys-X bonds, in agreement with earlier studies in other laboratories on plasmin specificity. The major site of cleavage was the Lys⁸⁹-Asn⁹⁰ bond in the middle of the polypeptide chain; however, cleavages also occurred to a considerable extent at other sites, in particular, the Lys⁵³-Arg⁵⁴, Lys¹³³-Ser¹³⁴, Lys¹⁵³-Leu¹⁵⁴, and Arg³¹-His³² bonds.

Significance to Biomedical Research and the Program of the Institute:

Knowledge gained from the studies described above are essential to an understanding of how the myelin sheath is assembled and maintained and, in addition, will provide insight into the nature of some of the pathological processes involving loss of myelin, in particular, plaque formation in multiple sclerosis.

Proposed Course:

We plan to continue our studies on the chemistry of the myelin basic protein, with emphasis on studies that will define more precisely its three dimensional structure in solution and its conformation in the myelin sheath. We wish to explore the nature of basic protein dimerization and the residues involved in the intermolecular contacts, since dimerization of the protein across the cytoplasmic surfaces of the myelin lamellae could be a mechanism for myelin compaction.

Publications:

- Mendz, G.L., Moore, W.J., and Martenson, R.E.: NMR studies of myelin basic protein. IX: Complete assignments of the tyrosine residues by proton NMR of proteins from six species. Biochimica et Biophysica Acta 748: 168-175, 1983.
- Law, M.J., Martenson, R.E., and Deibler, G.E.: Cleavage of rabbit myelin basic protein by thrombin. J. Neurochem. 42: 559-568, 1984.
- Martenson, R.E.: Myelin basic protein speciation. In Alvord, E.C., Jr., Kies, M.W., and Suckling, A.J. (Eds.): Experimental Allergic Encephalomyelitis: A Useful Model for Multiple Sclerosis. New York, Alan R. Liss, 511-521, 1984.
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Nygaard, E., Mendz, G.L., Moore, W.J., and Martenson, R.E.: N.M.R. of a peptic peptide spanning the triprolyl sequence in myelin basic protein. Biochemistry, in press.

Menz, G.L., Moore, W.J., Brown, L.R., and Martenson, R.E.: Interaction of myelin basic protein with micelles of dodecylphosphocholine. Biochemistry, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00901-29 LCM

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunologic Reactivity of Myelin Basic Protein

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: M. W. Kies Chief, Section on Myelin Chem. LCM, NIMH

Others: B. F. Driscoll Research Biologist LCM, NIMH

T. Fujii Guest Researcher LCM, NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Cerebral Metabolism

SECTION

Section on Myelin Chemistry

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS

2.5

PROFESSIONAL:

1.0

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

 (a) Human subjects (b) Human tissues (c) Neither (a1) Mixors (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

1) Studies on adoptive transfer of EAE in Strain 13 guinea pigs have yielded significant information on the cellular mechanisms which play a role in that experimental disease. Bacterial lipopolysaccharide (LPS) has been found to activate an essential pathway in the enhancement phenomenon which also depends upon exposure of the cells to specific antigen in culture. In the original studies LPS was unknowingly added to the culture medium as a contaminant in the fetal calf serum used to supplement the medium. (BP-sensitized cells, cultured with BP, are required but not sufficient for enhanced transfer of EAE).

2) Further studies on enhanced transfer of EAE in Lewis (Le) rats have developed some important information regarding differences among several mammalian basic proteins in their ability to induce in vitro proliferation and support enhanced transfer of EAE with BP-sensitized spleen cells. Results were correlated with known amino acid differences in the sequences of the encephalitogenic site in the different mammalian BPs.

3) Attempts to demonstrate EAE suppressor cells in Le rats protected against EAE induction have continued to be negative.

Project Description:Objectives:

Use of cell transfer to study immune mechanisms in the induction of EAE; specifically, to define the conditions under which culture with lipopoly-saccharide (LPS) activates BP-sensitized LNC cells; to define the specificity of the in vitro proliferative response; and to examine the suppressor cell theory for prevention of EAE.

Methods Employed:

Strain 13 guinea pigs and Lewis rats are sensitized with BP/CFA (specifically sensitized) or CFA (non-specifically sensitized). Lymph node cells (LNC) and/or spleen cells (Sp C) are removed from sensitized animals and used for passive transfer of EAE after the various manipulations indicated. Additions to culture (in the guinea pig/LPS experiments) and substitutions of different species-BP in the rat/species specificity study are used to manipulate EAE in the recipients.

Major Findings:

1) LPS requirement for transfer of EAE in Strain 13 guinea pigs - Successful adoptive transfer of EAE with BP-sensitized T cells cultured in vitro with specific antigen has been reported in several species (guinea pigs, rats, mice). We originally reported that BP-sensitized guinea pig peritoneal exudate cells (PEC) were markedly enhanced by culture with BP alone, but we recently discovered, serendipitously, that bacterial lipopolysaccharide (LPS) also plays an important role in culture. This discovery resulted, in part, from the observation that successful transfer of EAE was dependent upon the source of fetal calf serum (FCS) used in the culture medium. BP/PEC cultured with BP in medium containing FCS low in LPS (< 0.02 ng/ml) transfer EAE poorly. If exogenous LPS is added to the culture, good transfers are obtained with the same cells. Alternatively, severe EAE can be induced in the recipient, if aliquots of the cells are incubated separately, one with BP and one with LPS, and the two aliquots injected into the same recipient. The two cell populations interact synergistically in vivo to induce severe EAE.

If Cyclosporin A (CsA) is added to the aliquot of BP/PEC incubated with BP, transfer is prevented. On the other hand, addition of CsA to the aliquot of PEC incubated with LPS has no effect on the ability of the combined cell transfer to induce EAE. The cells which react with specific antigen (BP) are known to be T cells. In this report, we present data which suggests that the cells reacting with LPS are also T cells. They are present in the non-adherent population after C-10 column fractionation of PEC. While specific sensitization is not an absolute requirement for the LPS response, BP/PEC are far more effective than CFA/PEC in their ability to support transfer of EAE after exposure to LPS. Unlike BP activation, LPS activation does not involve cell proliferation.

2) Specificity of the rat encephalitogenic site in mammalian BPs - It is known that guinea pig BP is very encephalitogenic in Lewis rats while rat

BP is only about 1/10 as encephalitogenic on a comparable weight basis. Bovine BP is only slightly encephalitogenic (~ 1/50 as active as guinea pig BP). The cross-reactivity of the three BPs with respect to their ability to 1) enhance transfer and 2) induce a proliferative response in vitro has been examined with the following results:

<u>Sensitization</u>	<u>Active Induction</u>	<u>BP in Culture</u>	<u>Enhanced Transfer</u>	<u>Proliferative Responses</u>
GPBP	++(1)*	GPBP	++	++
"		Rat BP	+	+
"		Bov BP	±	-
Rat BP	+(1/10)*	GPBP	+	-
"		Rat BP	+	-
"		Bov BP	-	-
Bov BP	±(1/50)*	GPBP	-	-
"		Rat BP	-	-
"		Bov BP	-	-

* Relative activities

Sequence of Encephalitogenic site:

GPBP Leu-Pro-Gln-Lys-Ser-Gln-----Arg-Ser-Gln-Asp-Glu-Asn
 Rat BP Leu-Pro-Gln-Lys-Ser-Gln-----Arg-Thr-Gln-Asp-Glu-Asn
 Bov BP Leu-Pro-Gln-Lys-Ala-Gln-Gly-His-Arg-Thr-Gln-Asp-Glu-Asn

The structure of this site in the BP molecule influences the in vivo response to sensitization as well as the in vitro response of cells to the encephalitogen in culture (GPBP >> Rat BP >>> Bov BP).

3) Search for suppressor cells - In continuing attempts to understand the pathogenesis of EAE, we have searched for suppressor cells in animals protected against EAE. Cells were transferred from donors protected by injections of BP in incomplete Freund's adjuvant (IFA) to naive recipients. The latter were immediately sensitized with BP in complete Freund's adjuvant (CFA). Animals which received the putative suppressor cells were just as susceptible to BP-sensitization as those which received no cells or non-specifically sensitized cells. In attempting to explain lack of suppression we examined the efficiency of protection in the cell-donors. We showed that rats are more effectively protected by injections of BP in IFA if 1) they are 6 to 8 weeks old rather than 3 to 4 mo; 2) they are sensitized with antigen in adjuvant which contains *M. Butyricum* rather than *M. tuberculosis* and 3) they are challenged with barely effective rather than maximal doses of antigen. We then obtained cells from optimally protected donors and examined the ability of their cells to prevent induction. We still could find no evidence of suppressor or cells in the cell inoculum.

Significance to Biomedical Research and Program of the Institute:

This work represents an important addition to the study of pathogenetic mechanisms in the experimental disease, allergic encephalomyelitis. In the human idiopathic conditions thought to be caused by autoimmune reactions, the mechanism of sensitization, the effect of lymphokines on the reticuloendothelial system, and the initiation of the lesion in the target organ are all completely unknown. It is important to examine in vitro behavior of specifically-sensitized cells, to ascertain whether the proliferative response of helper cells is an essential requirement for lesion induction and whether these cells require an additional boost from a pathway totally independent of the proliferative pathway. Further, do suppressor cells play a pivotal role in cellular hypersensitivity by switching an "on" response to "off" or are they produced as a result of some other mechanism?

It has been thought for many years that multiple sclerosis may be an autoimmune disease. More recently, several investigators have suggested that Alzheimer's disease may also be the result of an adverse autoimmune reaction. It is important to work out possible mechanisms for the initiation and formation of CNS lesions in these conditions in order to provide a theoretical basis for treatment and/or prevention.

Proposed Course:

The most important problem at present is the identification of the non-specifically activated cells which interact synergistically in vivo with the BP-sensitized cells. These cells are probably responsible for producing inflammation at the site of a CNS lesion initiated by the BP-specific cells. This will be pursued not only by in vitro manipulations of these cells but also by comparing the histologic lesions of animals receiving various cell types.

Publications:

Richert, J.R., Kies, M.W., and Alvord, E.C., Jr.: Adoptive transfer of experimental allergic encephalomyelitis: Inhibition by agent that elevate intracellular cyclic AMP levels. Neurochem. Pathol. 1, 81-90, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00902-19 LCM

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Induction and Prevention of Experimental Allergic Encephalomyelitis (EAE)

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: B. F. Driscoll Research Biologist LCM, NIMH

Others: M. W. Kies Chief, Section on Myelin Chem. LCM, NIMH

COOPERATING UNITS (if any)

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LAB/BRANCH

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INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

3

PROFESSIONAL:

1.5

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Cells capable of adoptive transfer of experimental allergic encephalomyelitis (EAE) are manipulated in vitro in order to examine the mechanisms responsible for this autoimmune disease. Cellular immunity to myelin basic protein (BP) is required for induction of EAE. Cellular proliferation, IL-2 production and IL-2 utilization, are necessary but not sufficient for disease transfer. Other pro-inflammatory pathways must be induced if acute clinical EAE is to develop. Lipopolysaccharide (LPS) or LPS-activated cells apparently provide the necessary pro-inflammatory component and, in collaboration with BP-specific cells exposed to IL-2, transfer severe EAE. In addition to the cellular immunity and inflammation in acute EAE, chronic EAE has the added component of demyelination. Demyelination can now be induced in animals injected in separate sites with encephalitogen (i.e. BP) and an encephalitogen-free source of central nervous system antigens (i.e. chicken brain). The antigen responsible for demyelination is found in the myelin fraction of chicken brain.

Project Description:Objectives:

Dissection of the various cellular events involved in the pathogenesis of EAE, including antigen-specific immune responses and non-specific inflammatory responses; investigation of the antigen(s) responsible for, and the mechanism of, demyelination in chronic EAE.

Methods Employed:

BP-sensitized lymphoid cells from Lewis rats are exposed in vitro to either BP or various non-specific factors. These cells are then transferred alone or in combination with non-specific cells to naive recipients to assess their effectiveness in transfer of EAE. Chronic EAE is studied in Strain 13 guinea pigs made hyporesponsive to EAE induction by sub-optimal cell transfer and subsequently sensitized with guinea pig spinal cord in CFA or chicken brain and guinea pig BP in CFA.

Major Findings:

EAE consists of a complex series of sequential reactions - development of BP-specific cell mediated immunity, infiltration of the CNS with inflammatory cells, development of clinical signs of disease and, finally, demyelination in the CNS and persistent neurologic damage. These three separate areas (cellular immunity, inflammation and demyelination) all must be examined separately to understand a complex disease such as chronic EAE in guinea pigs or multiple sclerosis in humans.

Immunity to BP and CNS Inflammation - BP-sensitized cells from EAE susceptible Lewis rats transfer severe EAE to naive recipients after in vitro culture with BP. Reactions occurring during this culture include antigen presentation to BP-specific T cells, antigen-specific proliferation, and production of and response to various lymphokines. To analyze this situation, cells are not exposed to BP but are cultured with lymphokine preparations and their ability to transfer EAE is assessed. The lymphokine involved in cellular proliferation (IL-2), while necessary, is not sufficient to produce severe EAE in recipients. However, if recipients injected with these weakly reactive cells are also injected with non-specific cells previously exposed to LPS, severe EAE results. In this case, the lymphokine-activated, BP-sensitized cells provide a source of cell-mediated immune recognition of BP in the CNS while the LPS-activated cells are clearly required for a full-blown inflammatory response and the development of clinical EAE. Elucidation of the mechanisms by which the non-specific cells function is important. Even though they do not contribute to immunity to BP, they are predominantly responsible for the subsequent inflammation and, therefore, for the clinical signs of disease. A similar pathway, independent of IL-2 production and cellular proliferation, must occur in cultures of BP-sensitized cells with BP since these cells alone transfer severe clinical EAE.

There appears to be a natural model with which we have been able to demonstrate the dual roles of cellular immunity and inflammation leading to

clinical EAE. A group of Lewis rats (termed Lewis-resistant or LeR) develops cellular immunity to BP upon sensitization with BP in CFA, but fails to develop clinical signs of EAE. The cells from these animals proliferate in response to BP in vitro but do not transfer EAE after culture. Assuming that this reflects an intact cellular immunity to BP but a defect in development of inflammation, we added LPS to the culture of LeR BP-sensitized cells and BP. Cells recovered from these cultures transferred acute EAE to recipients. The mechanisms by which LPS stimulates transfer of disease are not known. Their identification is important because they are responsible for induction of clinical disease in cell recipients; furthermore, a defect in this pathway apparently allows the LeR rats to remain healthy even though they develop cellular immunity to BP. Once these pathways are identified, they could be manipulated to prevent disease in susceptible animals at a level separate from induction of antigen-specific immunity.

Chronic EAE - Chronic EAE is a more complex type of disease involving not only specific immunity to BP and inflammation but subsequent CNS demyelination. We reported that chronic, demyelinating EAE could be induced in adult strain 13 guinea pigs made hyporesponsive to BP sensitization. In this case, sensitization with whole guinea pig spinal cord served as a source of both BP (encephalitogen) and any antigens responsible for the induction of demyelination. We now have been able to separate these two factors by sensitizing guinea pigs with BP in one site and an encephalitogen-free source of CNS antigen in another. Chicken brain was chosen as the source of CNS antigens since chicken BP is non-encephalitogenic in strain 13 guinea pigs, yet all the other CNS antigens are present. Guinea pigs sensitized with BP and chicken brain develop large areas of demyelination in their CNS and frequently develop persistent neurologic signs typical of irreversible CNS damage. The antigen(s) responsible for demyelination have so far been localized to the myelin fraction. Further fractionation and identification of the antigens responsible for demyelination should be possible.

Significance to Biomedical Research and the Program of the Institute

A clear understanding of the events responsible for generation of an immune response is necessary before intelligent intervention in the process is possible. This is particularly true of cell-mediated immune responses, including DTH. Delineating the role played by specific immunity, subsequent inflammation and, in the case of the CNS, demyelination, is critical to our understanding of various pathologic states. While in vitro testing is useful for detecting specific immunity, studies on specific immunity leading to inflammation must be done in vivo. EAE provides an invaluable system for delineating these responses. Furthermore, chronic EAE is the best available animal model of CNS demyelination and, coupled with studies in immunity and inflammation, should prove a useful model of immunopathologic diseases of the CNS, especially multiple sclerosis.

Proposed Course:

We are attempting to delineate the various steps involved in development of clinical disease in EAE. In particular, it appears possible to separate development of cellular immunity to BP from the development of inflammation in

the CNS. Whereas LPS has been used to elicit cells capable of causing inflammation in BP-specific lesions, there undoubtedly is a natural lymphokine or monokine functioning in this respect in vivo. Identification of these products is critical since their activity is required for development of clinical disease.

By inhibiting this inflammatory response in clinical disease, it might be possible to prevent the development of demyelination. Disease activity in MS could be due to an inflammatory process but the major long-term damage in MS is demyelination. We have been able to separate the encephalitogenic component (BP) from the component responsible for demyelination and can now identify the latter. So far, the entity responsible for inducing demyelination has been localized to the myelin fraction of whole CNS tissue. By means of various biochemical fractionation procedures, the identification of the component of CNS tissue responsible for demyelination should be possible.

Publications:

Driscoll, B.F., and Kies, M.W. Expression of Ia antigen by cells responsible for enhanced transfer of EAE. In Alvord, E.C., Jr., Kies, M.W., and Suckling, A.J. (Eds).: Experimental Allergic Encephalomyelitis: A Useful Model for Multiple Sclerosis. New York, Alan R. Liss, 1984, pp. 291-298.

Fujii, T., and Driscoll, B.F.: In vitro stimuli required for enhanced transfer of experimental allergic encephalomyelitis with myelin basic protein sensitized guinea pig peritoneal exudate cells. In Alvord, E.C., Jr., Kies, M.W., and Suckling, A.J. (Eds). Experimental Allergic Encephalomyelitis: A Useful Model for Multiple Sclerosis. New York, Alan R. Liss, 1984, pp.299-305.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00903-07 LCM

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Identificaiton of Degradation Products Associated with Human Myelin Basic Protein

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: G. E. Deibler Chemist LCM, NIMH

Others: M. W. Kies Chief, Section on Myelin Chem. LCM, NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

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SECTION

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INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS

2.5

PROFESSIONAL:

1.5

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Human myelin basic protein (HBP) isolated from whole CNS tissue or from its myelin fraction contains two HBP-related polypeptides very similar in size and charge to the 18.5 K major basic protein. In our original isolation, we assumed these to be breakdown products arising during extraction. However, a modified and improved isolation procedure (described in last year's annual report) did not change the relative amount of these polypeptides (HBP and "fragments"). During the isolation, crude HBP is purified by ion-exchange chromatography. Fraction 1 (component 1, the last peak eluted from the column) was identified as the 18.5 K major basic protein. Fraction 3 (third from the last peak) was approximately 50% of the 18.5 K basic protein (minus 2 positive charges) and 50% other proteins which were smaller than HBP but had the same charge. Since it has been impossible to separate the 18.5 K major basic protein from the two polypeptide fragments by our present purification techniques (ion-exchange chromatography, molecular sieving and HPLC) we developed two new procedures - 1) tryptic mapping of thrombic fragments of HBP by HPLC and 2) electrophoretic transfer of peptides from 15% slab gels. By utilizing these two procedures plus amino acid analysis of the tryptic peptides, we were able to identify one of the smaller proteins in Fraction 3 as HBP minus the first 18 amino acid residues. The second small polypeptide in Fraction 3 has only been tentatively identified. These data confirm the fact that human myelin basic protein is mainly the 18.5 K major basic protein and two smaller "fragments" which we know are not products of our isolation procedure. These smaller "fragments" of HBP may be the result of autolysis in situ, and/or the result of a gene deletion.

Project Description:Objectives:

The purification and identification of the smaller polypeptides which are isolated with the major 18.5 K protein of HBP.

Methods Employed:

The new procedure for preparation of human myelin basic protein and our more sensitive electrophoretic system were both described in last year's annual report.

The standard method of tryptic mapping of basic proteins by thin layer chromatography (TLC) is less precise than isolation and analysis of individual peptides by HPLC. When basic protein (the 18.5 K protein) is digested by trypsin and the digest fractionated on HPLC it is difficult to separate the individual peptides for amino acid analysis. Our new technique involves the preparation of the two large thrombic peptides from purified BP. These peptides are separated easily and can then be subjected to tryptic digestion separately. A standard reverse phase HPLC method was developed for the N-terminal peptide (residues 1-97) and another method for the C-terminal peptide (residues 98-170). Each tryptic peptide can be eluted separately and identified by amino acid analysis. Once these standard chromatograms were established unknown basic proteins or peptides could be subjected to the same procedures and therefore, be identified by the peak elution times of their tryptic peptides. If the peak times differed from those of the standard, the unusual tryptic peptides were collected and identified by amino acid analysis.

Major Findings:

The new HPLC procedure for tryptic mapping is more rapid and conclusive than the old TLC method. Tryptic peptides that were not identifiable by the old technique can be easily resolved by this more sensitive method. It will separate peptides differing only by the different oxidation states of methionine in the peptide, as well as peptides containing the tri-proline sequence which differ only in cis or trans conformations. Also, monomethyl arginine-containing peptides are separated from dimethyl arginine and arginine-containing peptides. Much less protein or peptide is used for this technique than for the usual tryptic maps.

Since we were unable to separate the two smaller proteins from the major 18.5 K protein in Fraction 3 of HBP, we subjected the mixture to thrombic digestion. However, only one of the resulting five peptides could be purified by ion-exchange chromatography. Only partial purification was obtained by reverse phase HPLC. Electrophoretic patterns showed the 5 bands to be well separated electrophoretically. Therefore, we developed an electrophoretic transfer to purify the peptides. This technique involved running many slab gels, locating the peptides on each slab, cutting out the peptides separately and transferring them electrophoretically. Each transferred peptide was lyophilized and desalted. Then, the purified peptides were subjected to tryptic mapping by

HPLC as described above. The different tryptic peptides were identified by amino acid analysis.

One of the "fragments" in Fraction 3 of HBP has been identified as a possible autolytic breakdown product, residues 18-170. The second "fragment" is partially identified as HBP which has lost 10 or 11 residues preceding the tryptophan and another 15 residues on the C-terminal end of the molecule.

The new HPLC tryptic mapping procedure was also useful in the purification of tryptic peptides from pig. These tryptic peptides were then used for sequence analysis. In fact, the tryptic digest of any large peptide from any other species of myelin basic protein can be analyzed by this method.

Significance to Biomedical Research and the Program of the Institute:

Our ability to isolate highly purified HBP (component 1) allows us to prepare peptides of known sequence and study the biological activity of different regions of the molecule.

Since HBP extracted from isolated human myelin also contains degradation products, and since we now have a method for studying these products, we can attempt to determine their origin. The identification of these fragments may provide some insight into the abnormal metabolism which gives rise to BP peptides in CSF of patients suffering from demyelinating disease.

Proposed Course:

Because we have prepared HBP essentially free of enzymatic activity and have developed a new technique for tryptic mapping by HPLC, we can now investigate further the degradation products which are still present. We are in the process of developing an HPLC method for purifying these fragments. Once we have them pure we will be able to identify their sequence by HPLC tryptic mapping, N-terminal, C-terminal and amino acid analysis. Similar studies will be carried out on fragments prepared by incubation of the old batch preparation of HBP. A comparison of the two sets of fragments should enable us to determine whether the fragments which accompany HBP to the final purification step were associated with BP in situ or were created by autolysis. It is possible that the second smaller "fragment" associated with HBP (Fraction 3) arises from a gene deletion.

Publications:

Deibler, G.E., Boyd, L.F., Bacon, M.L., Driscoll, B.F., and Kies, M.W.: Purification by HPLC of a large peptic fragment of myelin basic protein. Neurochem. Pathol. 1: 1-15, 1983.

Deibler, G.E., Boyd, L.F., and Kies, M.W.: Proteolytic activity associated with purified myelin basic protein. In Alvord, E.C., Jr., Kies, M.W., and Suckling, A.J. (Eds.): Experimental Allergic Encephalomyelitis: A Useful Model for Multiple Sclerosis. New York, Alan R. Liss, 249-256, 1984.

Deibler, G.E., Martenson, R.E., Krutzsch, H.C., and Kies, M.W.: Sequence of guinea pig myelin basic protein. J. Neurochem. 43: 100-105, 1984.

Deibler, G.E., Boyd; L.F., and Kies, M.W.: Enzymatic and nonenzymatic degradation of myelin basic protein. Neurochem. Res., in press.

Others:

A. Bozzi	Visiting Associate	LGCB NIMH
G. de la Haba	Research Scientist	LGCB NIMH
D. Carotti	Guest Researcher	LGCB NIMH
T. M. Caryk	Chemist	LGCB NIMH
S. Scarpa	Biologist, Istituto Superiore di Sanita, Rome, Italy	
R. Strom	Biochemist, Istituto di Chimica Biologica, Univ. of Rome, Rome, Italy	

Project Description:

As is well known, S-adenosylmethionine (AdoMet) is a key intermediate in biological transmethylation and transalkylation reactions. There are hundreds of reactions, each catalyzed by a specific enzyme, that utilize AdoMet as a substrate. It is obvious that the utilization of AdoMet in biological systems must be under regulatory controls, but at the present time little is known about the nature of these controls. It has been established that S-adenosylhomocysteine (AdoHcy), one of the products of transmethylation reactions that utilize AdoMet as methyl donor, is a competitive inhibitor of most reactions in which AdoMet participates. From the result of work in this and other laboratories, it has been proposed that the intracellular ratio of AdoMet/AdoHcy must be of key importance in the regulation of biological alkylation reactions, and that this ratio plays a role in determining the hierarchy of biological methylation reactions. In eukaryotes, AdoHcy is metabolized through a single metabolic pathway by S-adenosylhomocysteine hydrolase (AdoHcyase), an enzyme which catalyzes the reversible hydrolysis of AdoHcy to adenosine and homocysteine. Because of the central role of AdoHcyase in the metabolism of AdoHcy and in maintaining the ratio of AdoMet/AdoHcy, this enzyme has been under intensive study in this and other laboratories.

Studies on purified AdoHcyase from several sources have shown that this is a complex enzyme, made up of four subunits, which bind NADH, adenosine and cAMP. Adenosylhomocysteine hydrolase of rat liver has been purified to homogeneity (2 dimensional electrophoresis). The following observations have been made: 1. The enzyme can be totally (and irreversibly) inactivated if preincubated with Mg·ATP·KCl, conditions which lead to optimal binding of cAMP. We have shown that this inactivation can be completely reversed if the inactive enzyme is incubated in the presence of NAD (but not NADH or NADP). NAD is a cofactor of the enzyme and a participant in the catalytic mechanism; the tetrameric enzyme contains 1 mole of NAD per subunit. We further showed that inactivation leads to the loss of all the NAD in the enzyme, and that reactivation is accompanied by the uptake of 3 or more moles of NAD/mole of enzyme. The enzyme is also inactivated by cAMP and can be reactivated by NAD. 2. It has been extensively reported that various nucleosides in addition to adenosine inactivate the enzyme because the bound NAD is reduced to NADH. We confirmed this using 2-deoxyadenosine, and showed further that the enzyme can be reactivated by NAD if the inactive enzyme is first subjected to gel filtration. This suggests that the NADH formed, unlike the NAD bound to the enzyme, is relatively loosely bound and may be removed by gel filtration. This experiment further suggests that it may be possible to protect the enzyme from inhibition by nucleosides (the basis of the toxicity in adenosine deaminase deficiency) if NAD is present in excess.

The purified enzyme has also been used to produce antibodies against the enzyme in rabbits. The antibodies will be used in future work for isolation of the mRNA for the enzyme and also for development of a radioimmune assay for determining levels of the enzymatic crude cell extracts.

While the biochemical mechanisms of transmethylation reactions have been elucidated many years ago, largely as a result of the studies by Cantoni and his collaborators at NIH, the correlation between many methylation reactions and cellular functions remains obscure. For instance, the significance of the methylation of a variety of informational macromolecules, such as proteins and nucleic acids (DNA, ribosomal-, messenger-, viral and tRNA, etc.), or of complex polysaccharides, or even simpler compounds such as guanido acetic acid, nicotinamide, etc., is not immediately obvious and is the subject of much debate. It can be surmised that modulation of AdoMet/AdoHcy ratio would result in important physiological effects, which if correlated with biochemical data would help reveal the significance of specific methylation reactions.

In prokaryotes, the isolation of mutants has helped to analyze the functions of specific biochemical reactions. In eukaryotes, isolation of mutants is more difficult, so other approaches have been devised, such as using inhibitors to block specific reactions. Since AdoHcyase is the only enzyme to metabolize AdoHcy in eukaryotes, inhibition of this enzyme by analogs can be used to alter the ratio of AdoMet/AdoHcy in the cell. We decided some years ago to take advantage of this fact and initiated a long range experimental project designed to study in depth the properties of AdoHcyase, and then to develop a series of specific inhibitors of this enzyme. As a result of these studies on the properties of AdoHcyase, we have established that the use of specific inhibitors makes it possible to alter the intracellular levels of AdoHcy and/or to accumulate intracellularly congeners of AdoHcy of the general formula S-purinyll-homocysteine (PurHcy). By using these inhibitors, it is possible to modulate the AdoMet/AdoHcy and/or AdoMet/PurHcy ratio in different cellular systems, and to examine the consequences of these changes on cellular functions.

Our studies, confirmed and extended in other laboratories, have shown that inhibitors of AdoHcyase may be divided into two groups: a) irreversible or suicidal inhibitors, and b) competitive inhibitors that inhibit the enzyme reversibly. This second group can be further classified into two subgroups; those inhibitors which can be utilized as substrates by the enzyme and those inhibitors which are not substrates. Several compounds have been examined which are irreversible inhibitors of AdoHcyase, and include the compounds 9- β -D-arabinofuranosyladenine (Ara-A), 3-deaza-9- β -D-arabinofuranosyladenine (3-deaza-Ara-A), and 2-chloroadenosine. Ara-A has been used by others in chemotherapy for cancer patients. 3-Deaza-Ara-A and 2-chloroadenosine might be expected to have clinical effects similar to Ara-A, since they produce similar inhibition of AdoHcyase. Of the many reversible inhibitors tested, two compounds have been extensively studied in this laboratory as prototype compounds of this group; 3-deazaadenosine (3-deaza-Ado) and 3-deazaaristeromycin (3-deaza-Ari). 3-Deaza-Ado is a potent competitive inhibitor of AdoHcyase with K_i of 5-8 μ M, and as a substrate it is about equivalent to the natural substrate, adenosine with a similar affinity for the enzyme. In contrast to 3-deaza-Ado, 3-deaza-Ari is not a substrate for AdoHcyase, but it is a very potent competitive inhibitor, with K_i of 2.0 nM for the hamster liver enzyme. Neither compound is a substrate for either adenosine kinase or adenosine deaminase.

In vivo, administration of 3-deaza-Ado to laboratory animals or cells in culture results in the accumulation of both 3-deazaadenosylhomocysteine (3-deaza-AdoHcy) and AdoHcy. The accumulation of 3-deaza-AdoHcy can be increased by addition of homocysteine, due to AdoHcyase acting in reverse of the normal hydrolytic direction. Administration of 3-deaza-Ari brings about an increase in AdoHcy, due to the inhibition of AdoHcyase. Since 3-deaza-Ari is a potent inhibitor of AdoHcyase, the amount of AdoHcy which accumulates, reflects the rate of transmethylation reactions, and not the catalytic rate of AdoHcyase. Differences between species were observed for the metabolite levels formed after treatment with these compounds. Administration of 3-deazaadenosine to rats produced accumulation of both 3-deaza-AdoHcy and AdoHcy, however, the same treatment in hamsters resulted only in an accumulation of 3-deaza-AdoHcy. Examination of the kinetic properties of the enzyme from rat and hamster liver revealed that the K_m for AdoHcy is ten times smaller for the hamster enzyme than for the rat. This could explain the lack of AdoHcy accumulation in hamster liver.

It would be expected that the intracellular accumulation of AdoHcy and/or 3-deaza-AdoHcy, with the accompanying changes in the AdoMet/AdoHcy ratio, would result in the inhibition of a number of transmethylases. This should cause an increase in the intracellular level of AdoMet (as a consequence of its under-utilization) and in a decrease in the intracellular concentration of many methylated intermediates. We have been able to verify this prediction, demonstrating a striking decrease in the amount of many methylated compounds, including methylated phospholipids, methylated proteins, and creatine in the liver.

Studies in this and other laboratories on a large number of analogs of AdoHcy analogs have demonstrated a wide range in the sensitivity of different trans-methylases to inhibition by these compounds in vitro. Unfortunately, cellular membranes are relatively impermeable to AdoHcy and its analogs, so it has been difficult to take advantage of the specificities of these analogs in vivo. However, the capacity of AdoHcyase to synthesize AdoHcy analogs in vivo, as has been shown with 3-deaza-Ado, demonstrates the exciting possibility of synthesizing potent and specific methylation inhibitors intracellularly.

Since 3-deazaaristeromycin has such a low K_i for AdoHcyase, high concentrations of this compound should effectively block the enzyme, preventing the conversion of AdoHcy to adenosine and homocysteine. Comparison of the effects of 3-deaza-Ado and 3-deaza-Ari on the replication of RAW264 cells showed that, at sufficiently high concentrations, 3-deaza-Ado was cytolytic after one day and that 3-deaza-Ari was cytostatic. Micromolar homocysteine reversed the cytostasis of 3-deaza-Ari, but did not reverse the cytotoxicity of 3-deaza-Ado. The cytotoxicity of 3-deaza-Ado is likely mediated by 3-deaza-AdoHcy, while on the other hand, cytostasis of 3-deaza-Ari was due to a profound inhibition of AdoHcyase. Since AdoHcy is the only cellular source of homocysteine, cells incubated with 3-deaza-Ari cannot recycle methyltetrahydrofolate and regenerate tetrahydrofolate for use in de novo synthesis of purines and pyrimidines. This condition is similar to the situation with vitamin B₁₂ deficiency, which inactivates methionine synthase, and causes methyltetrahydrofolate to accumulate. In addition, it would be expected that cells incubated with 3-deaza-Ari would contain less cystathionine, an amino acid without a known function that is found in high concentration in the brain. These findings could have clinical significance in situations where AdoHcyase is inhibited such as the administration of Ara-A and patients with adenosine deaminase deficiency.

Comparison of the biological effects of 3-deaza-Ado and 3-deaza-Ari has made it possible to attribute some of the differences in specificity to the finding that 3-deaza-AdoHcy is a more potent and specific inhibitor of some transmethylation reactions than AdoHcy. We have found that macrophage chemotaxis is specifically inhibited by the intracellular formation of 3-deaza-AdoHcy, brought about by treatment of the cells with 3-deaza-Ado, while chemotaxis is unaffected by accumulation of AdoHcy by treatment with 3-deaza-Ari. Both 3-deaza-Ado and 3-deaza-Ari bring about a significant inhibition of methylation of phosphatidylethanolamine in these cells, which rules out a requirement for this reaction in chemotaxis. In the same manner, both compounds inhibit protein lysine and arginine methylation and carboxylmethylation to the same extent. We have further shown that inhibition of chemotaxis by 3-deaza-Ado is correlated with inhibition of the synthesis of specific proteins which are not inhibited by 3-deaza-Ari.

Both 3-deaza-Ado and 3-deaza-Ari inhibit various RNA and DNA viruses, however, the sensitivity of various viruses to these two drugs is different. In addition, the synthesis of cellular mRNA in macrophage cells is inhibited to a greater extent with 3-deaza-Ado than with 3-deaza-Ari. The specific reaction(s) involved in inhibition of RNA synthesis has not been identified. The effect of both compounds may be useful for examining the role of various methylations in the synthesis and processing of different classes of RNA.

Both 3-deaza-Ado and 3-deaza-Ari can stimulate cell differentiation in a number of cell lines, suggesting that a methylation reaction may be involved in altering gene expression in differentiation. Again, there are differences between these two compounds in their ability to induce differentiation in different systems. In collaboration with Drs. Scarpa, Strom, and Bozzi, it was found that 3-Deaza-Ado will increase the rate of myoblast fusion to form myotubes when the cells are placed in a permissive fusing medium. In addition, non-fusing variants of myoblast cells, which normally do not fuse in a permissive fusing medium, will fuse when 3-deaza-Ado is added. The effect of 3-deaza-Ado and 3-deaza-Ari on differentiation in a myloid cell line has also been examined. Both 3-deaza-Ado and 3-deaza-Ari effectively stimulate the synthesis of globin in these cells to an extent comparable with known inducers of globin synthesis. Work from several other laboratories has suggested that DNA methylation may be involved in expression of specific genes. It is possible that 3-deaza-Ado may cause differentiation of these cells by inhibiting DNA methylation. However, since 3-deaza-Ado also inhibits a number of other methylation reactions, further work will be required to identify the mechanism for this effect of 3-deaza-Ado.

In a series of recent studies in Europe and in this country, it has been found that AdoMet, given parenterally to depressed patients produced rapid and remarkable improvement in the clinical picture. These studies indicate that AdoMet has approximately the same antidepressant activity as the standard tricyclics, such as imipramine, amitriptyline, etc. It is noteworthy, however, that administration of AdoMet is not accompanied by any toxic side effects, and thus, this mode of therapy may represent a considerable improvement over the therapeutic regimens currently in use.

The mechanism of action of AdoMet in depressive illness is unknown. It should be pointed out, however, that the dose of AdoMet found to be effective in the management of clinical depression (200-400 mg/i.v./day) is very small compared

to the daily flow of methionine through AdoMet. Human adults synthesize and metabolize about 20 millimoles of AdoMet/day, or 20-40 times the dose used in clinical trials.

Significance to Biomedical Research and the Program of the Institute:

Studies of the AdoHcyase and its inhibitors are important to understanding the regulation and function of biochemical transmethylation, and have possible clinical applications in the development of specific inhibitors for certain methylation reactions. Since AdoMet dependent methylation reactions are involved in the synthesis of so many compounds, including DNA, RNA, proteins, lipids, and neurotransmitters, the regulation of these reactions can alter many cell functions. Inhibitors of methylation reactions have been shown to affect cell differentiation, leukocyte chemotaxis, and virus replication. The possible clinical applications could be in the development of compounds for use in chemotherapy, immunosuppression, and antiviral drugs. Because of the important role of methylation in neurotransmitter synthesis, these compounds could have important effects on brain function as well.

Proposed Course of Research:

Studies on several inhibitors will continue in order to determine specific mechanisms of inhibition, and to determine correlations between inhibition of specific reactions and the physiological effects of these compounds. Much of the work will focus on methylation reactions involved in leukocyte chemotaxis, and in RNA and protein synthesis. The differences in inhibition of lipid and protein methylation by the various compounds will continue to be examined.

Publications:

Kim, I.-K., Zhang, C.-Y., Chiang, P.K., and Cantoni, G.L.: S-Adenosylhomocysteine hydrolase from hamster liver: purification and kinetic properties. Arch. Biochem. Biophys. 226: 65-72, 1983.

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DeBlas, A., Adler, M., Shih, M., Chiang, P.K., Cantoni, G.L. and Nirenberg, M.: Novel inhibitors of CDP-choline synthesis, action potential calcium channels, and stimulus-secretion coupling. Proc. Natl. Acad. Sci. USA., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00934-12 LGCB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Biochemical Basis of Narcotic Drug Action

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PROFESSIONAL:

2

OTHER:

1

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In the past year, opiate receptors have been purified to apparent homogeneity from the membranes of neuroblastoma x glioma hybrid cells. The receptors were first reacted with the newly developed affinity ligand, super-FIT, labelled with tritium to high specific activity. Purification was accomplished by lectin and antibody affinity chromatography techniques and preparative polyacrylamide gel electrophoresis. The purified material migrates with an apparent molecular weight of 55,000 - 58,000 depending upon the electrophoretic system. We have also purified the GTP binding protein, N_i, to homogeneity from bovine brain membranes. The material has been characterized by analytical ultracentrifugation, amino acid analysis, subunit composition studies and by its activity as a substrate for pertussis toxin and in reconstitution of opiate inhibition of adenylate cyclase in pertussis toxin treated cells.

We have also determined that the long term increase in adenylate cyclase activity resulting from chronic exposure to opiates is a reflection of a decrease in the cyclase associated GTPase activity, perhaps as the result of a modification of N_i. Receptor down-regulation, induced by highly efficacious agonists only, does not appear to be related to changed N_i function.

Other Investigators:

J. Barker	Chief, Lab. of Neurophysiology	LNP	NINCDS
W. F. Simonds	Pharmacology Research Associate		NIGMS
B. Tocque	Guest Worker	LGCB	NIMH
G. Milligan	Visiting Fellow	LGCB	NIMH
R. Sekura	Research Chemist	LDMI	NICHHD
T. Burke	Research Chemist	LC	NIADDK

Project Description:

Over the past several years we have been engaged in the characterization of the interaction between opiate receptors and adenylate cyclase in a cultured neuronal cell line, NG108-15. The cell has a large number of opiate receptors which function to inhibit adenylate cyclase and thereby lower cAMP levels. In analogy to the addictive process, the cells become tolerant to, and dependent upon, opiates after prolonged exposure. This adaptive response is due to a gradual increase in adenylate cyclase activity which serves to maintain normal cAMP levels in the continued presence of opiates. In the past year, we have made significant progress in our understanding of the detailed mechanism of some of these opiate actions. Moreover, work in this and other laboratories has shown that the cyclic nucleotide linked mechanism of opiate action is operative in brain as well as in the cultured cell system.

As a first step towards the purification and reconstitution of the cellular constituents involved in opiate action, we solubilized opiate receptors from several sources using the zwitterionic detergent, CHAPS. Receptors which reversibly bind opiate ligands with the appropriate specificity were isolated from membranes of NG108-15 cells, brain tissue (both rat and beef), and human placenta. Each of these receptor preparations behaves as a macromolecular complex of Stokes radius near 7 nm and contains protein as an essential constituent.

Rice, Jacobson and their colleagues have recently prepared a series of opiate ligands containing alkylating functions so that they might serve as covalent affinity labels of the receptors. We have studied the biological activities of these substances and found several of them to be uniquely selective irreversible ligands for either μ or δ receptor subtypes. We have prepared several of these substances in radioactively labelled form so that they may serve as tracers for receptor characterization and purification. The most useful of these compounds, to date, are fentanylisothiocyanate (FIT) which covalently binds to the (exclusively) δ receptors of NG108-15 membranes and super FIT [(+)-3-Methylfentanylisothiocyanate]. Super FIT is 5-10x more potent than FIT, can be prepared in active and inactive enantiomers and shows much less non-specific binding than does FIT. Interestingly, these materials behaves as agonists even when covalently bound. The radioactive materials bind specifically only to a 58,000 M_r glycoprotein. Because this binding is blocked by active opiates and not by their inactive enantiomers, we believe the protein to be the recognition subunit of the opiate receptor. It has been purified by a combination of wheat germ lectin chromatography, antibody affinity chromatography and electrophoresis. Antibodies to FIT have been prepared and purified by affinity chromatography.

These have been coupled to Sepharose and have been successfully exploited as an affinity matrix to effect a 200 fold purification of the labelled receptor subunits on super FIT. The protein has been purified approximately 20,000 fold over starting membranes and appears to be homogeneous.

The guanine nucleotides GDP, GTP and guanosine-5'(β,γ -imido)triphosphate inhibit binding of opiates and opioid peptides to receptors solubilized from neuroblastoma x glioma NG108-15 hybrid cells. The inhibition requires sodium ions and reflects a decrease in affinity of receptors for opioid ligands. These observations are consistent with the suggestion that solubilized receptors are complexes composed of an opiate binding protein and a guanine nucleotide regulatory component. Indeed, when such preparations are subjected to gel exclusion chromatography, opiate binding activity migrates together with the guanine nucleotide regulatory proteins of adenylate cyclase. Opiate inhibition of adenylate cyclase activity was shown to result from a more direct stimulation of GTP hydrolysis catalyzed by an inhibitory receptor coupled GTPase in neuronal membranes. Neither opiate stimulation of the GTPase nor inhibition of adenylate cyclase is observed in detergent solutions of neuronal membranes. Thus, even though receptor binding in such solutions is GTP sensitive, some component of the receptor-GTPase coupling mechanism has become limiting. A major goal of our work is the reconstitution of coupling of receptor occupancy to adenylate cyclase inhibition (and GTPase stimulation) in solubilized preparations. Functional reconstruction of the coupled activities is necessary to determine the numbers of essential constituents and the role which each component plays. A first step towards reconstitution is our recent demonstration that functional opiate receptors can be transferred, by polyethylene glycol induced fusion, from membranes of NG108-15 cells to those of other cells.

Solubilized receptor preparations have not yet been reconstituted in this way. We have, however, recently had some success in incorporating solubilized receptors into phospholipid vesicles and at the same time maintaining both binding activity and GTP sensitivity of binding. Fusion of such active liposome preparations with the appropriate adenylate cyclase-membrane preparation should, we believe, lead to reconstitution of coupling. Experiments designed to test this hypothesis are currently in progress.

It has recently become clear that there are two GTP binding regulatory proteins associated with coupling receptors to adenylate cyclase. These are N_G which modulates stimulatory (β -adrenergic or dopamine, for example) receptor activity and N_I which modulates the activity of inhibitory receptors. These regulatory proteins are modified both in structure and function by the bacterial toxins causing cholera (N_G) and pertussis (N_I). We have developed assays for N_I activity based upon reconstitution of opiate receptor affinity and activity with pertussis toxin treated membranes, and have now purified N_I to homogeneity from beef brain membranes (a particularly rich source). This protein has so far been characterized by its stoichiometric ADP ribosylation with pertussis toxin, size and shape studies in the analytical ultracentrifuge and amino acid composition. It has also been possible to reconstitute opiate receptor - N_I interactions by adding purified N_I to pertussis toxin treated membranes.

Several years ago, we showed that cells accommodate to chronic exposure to opiates by developing an increased adenylate cyclase activity. This homeostatic mechanism is elicited by all opiate agonists. In addition, we have recently found that

highly efficacious agonists, such as etorphine or enkephalin, also cause a down regulation of receptor numbers whereas morphine does not do so. Down regulation of receptors is thus not necessary for homeostasis but occurs with some opiates only. Changes in N_1 function are not responsible for down regulation because other inhibitory receptors, such as α_2 -adrenergic, are unaffected by etorphine treatment. We have recently refined our assay procedures so as to be able to measure adenylate cyclase and GTPase activities simultaneously with membranes prepared by a rapid isolation procedure. We have found that the increased adenylate cyclase activity resulting from chronic opiate treatment reflects a stable modification GTPase activity.

Significance to Biomedical Research and the Program of the Institute:

A major problem in biology is understanding the mechanism of signal-response coupling across cell membranes. Cells communicate with one another and with their environment largely through chemical messengers which are sensed by cell surface receptors and thereby elicit other chemical changes within the cell. The opiates, and related substances, are important transmitters of information in the nervous system. An understanding of how brain cells transmit and use such information is essential to the design of rational therapy for mental illness.

Proposed Course of Research:

We plan to prepare enough purified opiate receptor to be able to produce antibodies, both conventional and monoclonal, to this material. Those antibodies will be used as structural and histochemical probes and in gene cloning experiments. We expect also to determine at least partial amino and sequences of the material. We will also continue our efforts to reconstitute receptor function from purified compounds in solution. Rigorous characterization of the properties of the isolated receptors and of N_1 will be carried out to aid our reconstitution efforts and our general understanding of receptor coupling mechanisms.

Publications:

Zioudrou, C., Varoucha, D., Loukas, S., Streaty, R.A., and Klee, W.A.: Photolabile opioid derivatives of D-Ala²-Leu⁵-enkephalin and their interactions with the opiate receptor after photolysis. J. Biol. Chem. 258: 10934-10937, 1983.

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Loukas, S., Varoucha, D., Zioudrou, C., Streaty, R.A., and Klee, W.A.: Opioid activities and structures of α -casein derived exorphins. Biochemistry 22: 4567-4573, 1983.

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Klee, W.A., Koski, G., Tocque, B. and Simonds, W.F.: The mechanism of receptor mediated inhibition of adenylate cyclase. Adv. Cyclic Nucleotide Res., in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00935-17 LGCB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Effects of Viruses and Plasmids on the Biochemistry of Living Organisms

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1

PROFESSIONAL:

1

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The primary goal of this study is to examine the role of viral integration and plasmids in Alzheimer's disease and the process of aging and senescence. Recent development of techniques to detect chromosomally integrated viral genomes had encouraged us to apply these methodologies to investigate human diseases. The possibility of an association between a viral genome integrated into the chromosome and the occurrence of diseases inherited in a dominant manner, such as familial Alzheimer's disease, is currently being examined.

Integration of viral related nucleotide sequences in the genomic DNA of man is being studied using fibroblast cell lines established from individuals belonging to a family with histologically confirmed Alzheimer's disease. Tissues from this Alzheimer pedigree are also being utilized along with tissues obtained from young and aged normal individuals to examine amplification and excision events in the mitochondrial genome as a function of the aging. Since excision can result in the synthesis of circularized mitochondrial plasmids, (the major factor in senescence of some fungal strains) experiments are being conducted to screen for these free mitochondrial plasmids and an attempt is being made to determine their relationship with the state of senescence.

Project Description:

The methodologies for studying viral genes and their expression in host cells have developed rapidly over the past decade. The extension of these methodologies to study dominant genetic diseases, such as familial Alzheimer's disease, for viral genetic information is utilizing restriction endonucleases, electrophoresis, and DNA-DNA hybridization. At the present time, skin biopsies from almost 70 individuals belonging to a family with histologically confirmed Alzheimer's disease have been taken. These biopsies have been used to establish fibroblast cell lines which now have been grown up in sufficient quantity to allow extraction of human DNA for each line.

These DNAs are being screened with [³⁵S]-labelled viral probes, which are synthesized from the nucleic acid of selected viruses. The viruses used include: human - herpes virus, adenovirus, poliovirus, measles virus, cytomegalovirus, Epstein-Barr virus and BK virus; primate - SV40, baboon type-C virus, and simian sarcoma virus; and murine - Rauscher murine leukemia virus, Moloney murine sarcoma virus and mouse mammary tumor virus. These viruses were selected for their potential to detect related sequences in the human genome. The choice of human viruses is obvious as is the selection of a few primate viruses on the basis of the evolutionary relatedness of the two species. Murine viruses are also included because of the finding of 11-28% nucleic acid homology between some primate and murine viruses.

Hybridization of a viral probe with a DNA restriction fragment may indicate the presence of virally related sequences in the cell lines. Since the fibroblasts were originally obtained from individuals comprising an extensive pedigree of familial Alzheimer's disease, the presence of chromosomally integrated viral genetic information could then be followed for genetic transmission throughout the family.

Many mammalian species contain endogenous viral information which usually remains "silent" due to either repression of the virus by the cell or integration of only a portion of the viral genome. The presence of these endogenous viral sequences in a wide range of animal species indicates their evolutionary preservation, and according to some, such genetic information might provide functions with a selective advantage to the species possessing them. These endogenous viral genes also have the potential to be turned on by environmental agents or derepressed in an aging cell resulting in cell transformation and for these reasons it is imperative to examine the human genome for sequences related to these viruses.

Previous studies have looked for homology between DNA extracted from a cell and the DNA of a viral probe by reassociation kinetics. Using these methods, free viral nucleic acid could not be distinguished from integrated viral sequences. However, new techniques involving restriction endonuclease digestion, Southern blotting (Southern, 1975) followed by hybridization of a viral probe, allow this project to examine whether the cellular DNA of man contains integrated virus related sequences. Additionally, the sites of integration can also be examined. Integration sites may prove to be useful as polymorphic markers in the mapping of the genetic loci on the human chromosomes. This information may also aid in the understanding of gene regulation.

A number of disease-associated tissues have been examined for the presence of human viral sequences. Poliovirus type I and type II were used to study amyotrophic lateral sclerosis (Miller et al., 1980) with negative results. Human cytomegalovirus (CMV) was used to probe DNA from the brains of patients with schizophrenia (Aulakh et al., 1981) and multiple sclerosis (Aulakh et al., 1980). Both of these hybridization analyses failed to detect any virus related genetic information complementary to the CMV probe. Herpes simplex virus (HSV) has also been used extensively to search for viral nucleotide sequences in brain tissue from patients with multiple sclerosis (Aulakh et al., 1980), idiopathic Parkinson's disease (Wetmur et al., 1979), and senile and presenile dementias of Alzheimer and Pick (Middleton et al., 1980), again with negative findings. Neoplastic tissue has also been analyzed with several viral probes. One hundred and sixty-six tumors representing about 50% of all cancer types in the United States were extracted by Wold and his colleagues (1978) and hybridized with BK virus, a human papovavirus. They were unable to detect BKV sequences in the DNA from any of the human tumors. However, these studies were all done using liquid DNA-DNA hybridization which is not as sensitive as the techniques being utilized in this project. One study which employed Southern blotting (a technique we are using) of human tonsil DNA digested with Eco RI followed by filter hybridization with group human adenoviruses (Green et al., 1979) found that all tonsils showed restriction fragments which might indicate adenovirus sequences integrated into tonsil DNA. Other investigations which indicate positive results include the identification of integrated hepatitis B virus DNA in a human hepatocellular carcinoma cell line Chakraborty et al., 1981).

Based on this wealth of conflicting data, it seems imperative to approach this concept with the latest techniques. Alzheimer's disease is a good candidate for such research since evidence has implicated incomplete, latent or unconventional virus infections as a possible primary pathogenetic event. Possibly the disease state is a result of age-dependent relaxation of gene repression which could lead to the expression of specific viral genes not normally expressed. This relaxation of gene control has been previously suggested in diseases which may be associated with slow viruses.

Given that endogenous viruses have been found in a wide variety of vertebrate species, such as reptiles, birds, rats, mice, pigs, cats, and primates, it seems likely that this project will uncover the presence of some virally related nucleotide sequences in the genomic DNA of man. Detection of integrated viral genes in human DNA will be of major importance in many fields of research and may spark increased analysis of virus associations in disease states.

Another approach to the study of diseases associated with aging, such as Alzheimer's, has been suggested by the work done with senescence in fungi. It appears that in fungi, senescence is caused by mutations affecting extrachromosomal genetic factors. This conclusion was drawn from three lines of evidence. The first evidence derives from the transmission of senescence in crosses of Podospora (Ricet, 1957; and Marcou, 1961). These researchers found that senescence is transmitted by the female parent since senescent male clones crossed with normal clones produced normal progeny, whereas, when the female parent was senescent, both normal and senescent progeny resulted. This pattern indicates that the genetic factor for senescence in fungi is not present in the nuclear chromosomal material but must be located in the cytoplasm of the cell.

The second line of evidence for the extrachromosomal location of senescence is derived from their "infectious nature." Normal clones of Neurospora become senescent following a transient fusion with senescent clones without acquiring any nuclear genes from the senescent strain (Bertrand and Pittenger, 1969). The state of senescence can also be transferred in Podospora without the relocation of nuclei or nuclear material.

The final and most convincing evidence was provided by microinjection experiments with Neurospora (Garnjobst et al., 1965; and Diacumakos et al., 1965). When normal clones were injected with purified nuclei or DNA from senescent cultures, no signs of senescence appeared. However, when the injected material was composed of cytoplasm or purified mitochondria from senescent cells, many of the resultant clones became senescent following several days of growth. These studies illustrated not only the extrachromosomal nature of the genetic factor but indicated that senescence is associated with the mitochondria. A model has been proposed for fungi, in which, the senescent state is triggered by mutational events which affect the mitochondrial genome. These defective mitochondria then multiply during the progression of senescence and eventually outnumber the normal mitochondria which leads to vegetative death.

Recent research into senescence in fungi has substantiated the proposed model and illuminated the specific mutational event which appears to initiate symptoms of senescence. Vierny and her colleagues (1982) have discovered a 2.6kb region in the mitochondrial genome of Podospora anserina which undergoes amplification. This sequence may also be excised out of the genome and exist as a free circularized sequence or plasmid. At the final stage of senescence, these plasmids constitute virtually all of the DNA found in senescent mitochondria. Mutant strains whose mitochondrial genomes lack the 2.6kb region display an increased longevity and may actually escape senescence. Free plasmids have also been found to migrate to the nucleus and become integrated into the nuclear genome (Wright and Cummings, 1983). This process might then promote instability and degeneration of the nuclear chromosomes.

The relationship between aging and changes in the mitochondrial genome is beginning to be investigated in more complex species. Mitochondrial DNA isolated from liver mitochondria from young and aged rats by cesium chloride, ethidium bromide isopycnic density gradient centrifugation revealed a novel band in 75% of the older animals (Murray and Balcavage, 1982). In eukaryotic cells, the occurrence of complex forms of mitochondrial DNA have been observed in electron microscopic preparations by many investigators (Clayton et al., 1968; Clayton and Vinograd, 1969; Wolstenfrolme et al., 1973; Pasletti and Risu, 1973). This abnormal mitochondrial DNA is present in two forms; circular dimers, and catenated dimers and higher oligomers. The catenated forms of mitochondrial DNA appear to be ubiquitous in isolates of mitochondrial DNA from a wide variety of eukaryotic cells and animal tissues, with frequencies ranging from 4-8% (Clayton and Smith, 1975). In contrast, the circular dimers are found at a much lower frequency. In adult mouse tissues, their frequency was 0.05% (Pokó and Matsumoto, 1977). However, the occurrence of these complex forms of mitochondrial DNA have been shown to change with age. Pikó and Matsumoto (1977) found an increase in both circular dimers and total complex forms in the tissues, especially the brain, of senescent mice. At this time, however, the mechanism

of formation and maintenance of these novel forms and their precise function in the cell is a major question.

Human cell lines and tissues (especially the brain) are being examined for mitochondrial mutational events associated with aging and senescence. The tissue mitochondrial DNA is purified, digested by restriction enzymes and analyzed for amplification or excision events. The cytoplasmic fraction is screened for free plasmids which may have originated in the mitochondria. DNA extracted from whole tissues or cells are being probed with sequences cloned from young mitochondria to determine if nuclear integration of plasmids is a factor in the senescence of humans.

Currently, we are in the process of cloning mitochondrial DNA sequences derived from human tissues. These cloned sequences will be useful to probe the DNA extracted from human tissues representing various age groups. Additionally, they can be used to examine the large family pedigrees that have been established in cell culture.

We have examined the DNA isolated from purified mitochondria on agarose gels and have observed several bands in addition to the 16.5 kb band that corresponds to mitochondrial genome. Three of these bands are of higher molecular weight and appear to represent dimeric, trimeric and tetrameric forms of mitochondrial DNA. The frequency of these forms across various age groups and tissue types has yet to be examined.

Several lower molecular weight bands were also observed in the preparations from human brain tissue. The nature of these bands and their frequency has not been investigated to date. However, should the process of senescence in mammalian species resemble the model proposed for fungi, we have also obtained the senescent plasmids of Podospora anserina from David Cummings, of Colorado, for use as potential probes.

Significance to Biomedical Research and the Program of the Institute:

Molecular genetic studies will prove to be a key to the understanding of normal brain function and the pathogenesis of neuropsychiatric diseases. There are a number of genetic neuropsychiatric diseases for which trait-specific molecular markers are lacking and for which the pathogenesis is largely unknown. Prominent among these is familial dominant Alzheimer's disease, the major focus of this project.

Because the prevalence of severe dementia in the Northern European population of age 65 or older is 4-5% and the prevalence of milder dementia is 11-12%, more than 1 million Americans are probably afflicted with severe dementia and 3 million have mild or moderate dementia. Cognitive dysfunction in old age does not occur inevitably; it is a consequence of pathological processes. Of demented patients, 50% show the neuropathological changes of Alzheimer's disease: neurofibrillary tangles, senile plaques and granulovacuolar bodies.

The diagnosis of Alzheimer's disease is presently clinical. Other causes of chronic cognitive deterioration such as toxins, nutritional deficiencies, infections, endocrine disturbances, cerebral tumors, arterial disease and normal-

pressure hydrocephalus must be ruled out. In elderly patients, depression frequently masquerades as dementia. Definitive diagnosis currently depends on the histological study of brain tissue. Brain neurochemical findings have stimulated investigators to attempt treatment by manipulating cholinergic neurotransmission and approaches might be made to the treatment of other dementias. There is a definite need for a better method for diagnosing Alzheimer's disease.

The cause of Alzheimer's disease is unknown. It may be a slow infectious agent such as scrapie in sheep or Kuru and Creutzfeldt-Jakob disease in man. Psychiatric and neurologic sequelae may follow years after viral infection (as with measles and subacute sclerosing panencephalitis and with influenza and postencephalitic Parkinson's disease). The paired helical filaments of neurofibrillary tangles and neuritic plaques in Alzheimer's disease could be due to the interaction of viral protein with cell cytoskeletal protein which occurs during viral replication. A factor from brain and Alzheimer patients assembled neurofilaments into paired helical filaments and was cytopathic in neuronal cell cultures. The factor was RNase and protease sensitive but DNase resistant. Brain suspension from patients with familial Alzheimer's disease showed cell-fusing activity in 59% of cases, a level similar to that for Creutzfeldt-Jakob disease; only 3 of 17 sporadic Alzheimer cases (17%) showed fusion activity. Like the Moloney leukemia virus, in the experiments of Rudolph Janish, the putative virus or other infectious agents might integrate into the human germ cell genome, be transmitted in autosomal dominant fashion and show tissue specific expression of effects. We are searching for viral sequences as genetic markers and as potential disease causative agents.

The strongest evidence for a genetic factor in Alzheimer's disease is the fact that all individuals with Trisomy 21 develop the brain pathology of Alzheimer's disease if they live to their late 20's or 30's. Based on this fact, our laboratory has approached the molecular basis of Alzheimer's disease by attempting to identify (using high resolution protein two-dimensional electrophoresis) proteins coded for or modulated by Chromosome 21.

Integrated viral sequences are not only of interest in themselves but may often behave as polymorphisms. Molecular polymorphisms are genetic variants with an allelic frequency of greater than 1% in the normal population, and serve as diagnostic markers and molecular probes. When a polymorphic locus is close to a disease locus, the two loci will rarely be separated by recombination and linkage may be established. Mathematical analysis indicates that with the current clinical polymorphic markers available (about 30) approximately 20% of the human genome is currently covered at a genetic linkage distance of 10 centimorgans. Discovery of a sufficient number of polymorphic marker loci will allow the construction of a high resolution map of the human genome. Such a map will allow most genetic diseases which are primarily monogenic to be localized to a genomic subregion and will provide additional molecular probes for investigations of pathogenesis.

Studies on the role of mitochondria in diseases associated with aging may provide needed insight into the process of senescence. Every organism undergoes the progression of aging, but little is known about the events which trigger the decline. Recent investigations have indicated that mutations in the mitochondrial DNA,

especially in the brain, may be associated with aging. Such changes in the critical energy supplying organelle of a cell are quite capable of resulting in profound effects.

The model proposed for the fungi may be simplistic when compared to the complexity of mammalian systems. However, considerable evidence has been accumulated on involvement of the mitochondrial genome in aging in mammals. The nature of this involvement; whether it is causative, predictive or a result of the state of senescence, needs to be examined. Such knowledge can then be used to explore the age-associated diseases and the various diseases characterized by senility.

Proposed Course of Research:

We plan to utilize the tissue and cell lines established from Alzheimer pedigrees and other large pedigrees with familial disorders to identify genomic localization of viral sequences. The precise nature of the integrated viruses will be examined as well as potential disease associations. If this approach using the most current molecular biological techniques is promising, it will be extended to the investigation of other neuropsychiatric disorders.

The work will also examine the role of plasmids derived from the mitochondrial genome in the process of aging. Using tissues from young and aged individuals, the mitochondrial genome will be examined for evidence of amplification or excision of specific regions. The nuclear chromosomal DNA from tissue of aged persons and from fibroblast cell lines established from members of the Alzheimer's disease family will be screened for the integration of senescent plasmids. The occurrence of these events will be studied in their relationship with the symptoms of aging and the diseases associated with aging.

Publications:

None

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00936-21 LGCB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Homocystinuria: Methionine Metabolism in Mammals

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

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0.5

PROFESSIONAL:

0.5

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

An international survey of patients with homocystinuria due to cystathionine β -synthase deficiency has been completed. Data upon 629 patients have been analyzed and submitted for publication. The results obtained increase knowledge about the prevalence and time of onset of each of the major clinical manifestations of this disease: mental retardation, thromboembolic accidents, dislocated optic lenses, convulsions, osteoporosis, and early mortality. These results provide baselines adequate for design of studies of effects of therapies and for interpretation of these studies even in the absence (for ethical reasons) of control groups of patients. B₆-Therapy for responsive patients or methionine restriction are demonstrated already to effectively prevent specified complications. Data on reproductive performance, effects of maternal homocystinuria, and occurrence of post-operative thromboembolic complications are also presented.

Project Description:

Within the general framework of investigating methionine metabolism in mammals, with emphasis on the human genetic diseases which result from abnormalities affecting this area of metabolism, efforts this year have focussed upon a questionnaire survey of patients with homocystinuria due to cystathionine β -synthase deficiency. The purpose of the present survey is to gather sufficient information in a standardized format so that some of the major uncertainties about the natural history of this disease, about the role of genetic heterogeneity, and concerning the use and effectiveness of various therapies may be assessed.

This survey was undertaken to clarify many of the major aspects of the natural history of homocystinuria due to cystathionine β -synthase deficiency, especially those which require statistical treatment of large patient samples, and which provide baselines for evaluation of therapy. At the time of last year's report, data collection and entry into computer format had almost been completed. Preliminary computer programs had been developed for major areas of data analysis. During the past year this project has been brought to virtual completion by finalizing data collection and entry, by development of additional required computer programs, and by preparation and submission for publication of a manuscript detailing the results of this project. Since the results reported in the last annual report were still preliminary and based upon incomplete data, the major results of the survey will be summarized here in more final form.

Data were obtained for 629 patients. We are aware of some 75-100 patients for whom data could not be obtained. Although the patient sample may have been subject to bias in several ways, such as lack of inclusion of mildly affected, undiagnosed patients, or under-reporting of severely affected, deceased patients, it appears that most cystathionine β -synthase deficient patients known to physicians in North America, Western Europe, Australia, New Zealand and Japan have been included.

As a first approach to controlling for the extensive genetic heterogeneity known to occur in the mutations producing cystathionine β -synthase deficiency, patients were divided according to their B_6 -responsiveness into the following categories: responsive (231 patients), non-responsive (231), intermediate (67), or unknown (100). This is admittedly an oversimplification, but is as far as one can go with the information currently available for most patients. In practice, B_6 -responsiveness or non-responsiveness turned out to be concordant within sibships, providing evidence that this classification has a genetic basis. Further, classification in this manner did result in statistical differences between B_6 -responsive and B_6 -non-responsive patients with respect to the severity of most of the major clinical manifestations.

Major new findings regarding each of the major clinical manifestations of cystathionine β -synthase deficiency may be summarized as follows:

a) Mental capability. There is a wide range of mental capabilities in cystathionine β -synthase deficient patients, with extensive overlap in this regard between B_6 -responsive and non-responsive patients. Nevertheless, there is clearly a tendency for B_6 -responsive patients (mean IQ, 79) to be less severely

affected than B₆-non-responsive (mean IQ, 57). Virtually all patients with IQ's of 90, or above, are B₆-responsive.

Many of the patients in the survey sample had mental retardation as at least one factor contributing to their ascertainment, raising the possibility that ascertainment bias influenced quantitatively the apparent extent of the mental impairment caused by cystathionine β -synthase deficiency. Comparison of patients ascertained by complete sibship screening with those in the proband group confirmed that ascertainment bias did exert such an influence. To estimate the maximum contribution of ascertainment bias, relevant analyses were repeated after prior removal of patients for whom mental retardation was a factor in ascertainment. The difference in IQ's between B₆-responsive and non-responsive patients remained, with the median IQ's for B₆-responsive and B₆-non-responsive patients each increasing by eight points.

b) Optic lenses. Time-to-event curves for lens dislocations in large, untreated groups of both B₆-responsive and B₆-non-responsive patients are reported for the first time. Both curves display a lag during the first two years of life, but thereafter there are statistically significant differences in the rates of lens dislocation between the two groups. For example, at age 10 years, chances of dislocation were 55 and 88% respectively. These curves also confirm that eventually the great majority of cystathionine β -synthase deficient patients develop dislocated lenses, as shown by the probability that only 3 percent of all such patients will have lenses in place by age 39 years.

Ascertainment bias might be particularly marked for ectopia lentis, which was the sole feature leading to ascertainment of almost 21% of patients discovered because of clinical stigmata and a contributory factor in an additional 65%. However the time-to-event curves for lens dislocations in late-detected probands did not differ significantly from the corresponding curves for those ascertained by complete screening of all sibs, providing limited assurance that the results reported for ectopia lentis were not severely affected by ascertainment bias.

c) Thromboembolic events. Time-to-event graphs for initial thromboembolic events in the untreated state are presented. These provide for the first time data based upon sufficiently large numbers of both B₆-responsive and B₆-non-responsive patients that realistic estimates can be made of the chances of suffering a clinically detected event of this sort over given age intervals. Again, the curves were significantly different for B₆-responders and non-responders (e.g. at age 15, chances of having had such an event were 12% and 27%, respectively). The cumulative probabilities of having thromboembolic events are lower than might have been expected from the literature. Both B₆-responders and B₆-non-responders have about a 70% chance of reaching age 20 years free of clinically apparent thromboembolic disorder.

Since early thromboembolic events were the sole cause of ascertainment in relatively few patients, and the time-to-event curves for first events were not significantly different for patients discovered by complete sibship screening and probands, there is little indication that ascertainment bias affects importantly the results reported for such events.

d) Osteoporosis. Time-to-event graphs are presented for detection of radiographically demonstrated spinal osteoporosis. At age 15, the chances of such osteoporosis having been detected were 36% for B₆-responders and 64% for B₆-non-responders, a significant difference. These curves are regarded as setting upper limits for the ages at which this condition is present in given proportions of patients. Osteoporosis could have been present, yet gone undetected until patients were ascertained for other reasons, and better serial data for this manifestation will be required to provide baselines which permit satisfactory evaluation of the effects of therapies.

e) Mortality. The survivorship data from the present survey indicate mortality rates much less rapid than those reported previously. Various analyses indicate that this is almost surely due to biasing of earlier patient samples toward more severely affected individuals. The actual prognosis for patients with cystathionine β -synthase deficiency is thus likely to be far better than had been thought previously.

f) Reproductive performance. The majority of full-term pregnancies in cystathionine β -synthase women resulted in apparently normal children. In particular, abnormalities similar to the mental retardation, microcephaly, congenital heart disease, and low birth weight often observed in the offspring of phenylketonuric women were not reported. The evidence is inconclusive whether untreated maternal cystathionine β -synthase deficiency leads to excessive fetal loss. The data for cystathionine β -synthase deficient men indicate that the in utero risk to the fetus from heterozygosity for this defect is minimal.

Treatment. A major aim of the present survey was to provide sufficient data upon the natural history of untreated cystathionine β -synthase deficiency so that the effects of treatment could be more satisfactorily evaluated in the future. In large part this aim has been achieved, and the baselines provided now set the stage for design and evaluation of future work, even if, for ethical reasons, control, placebo-treated patients can not be included in such studies. Data on treatment are presently insufficient to clarify many issues, but the following points can now be made:

Methionine restriction, usually accompanied by cystine supplementation, was the first treatment devised for cystathionine β -synthase deficient patients, and, for B₆-non-responsive patients, far more experience has been accumulated with this treatment than with any other. The results of this survey clearly indicate the efficacy of such therapy in preventing mental retardation in B₆-non-responsive patients who are detected as newborns and started early upon therapy. An improvement of the order of 35 points in mean IQ was observed in such patients. Such therapy may also decrease the rate of lens dislocations, and reduce the incidence of seizures. More definitive information on these manifestations will be acquired in the near future as further experience with these patients accumulates. Likewise, it is still too early to assess the effect of early onset methionine restriction therapy on thromboembolic events, mortality, or osteoporosis.

Methionine restriction, sometimes accompanied by pyridoxine therapy, has also been used for the majority of patients detected as newborns who are B₆-respon-

sive. The limited data available indicate that most subjects will attain normal or near normal intelligence when so treated.

Methionine restriction has apparently not produced statistically significant benefit upon lens dislocation or the rate of occurrence of first thromboembolic events in late-detected B₆-non-responsive patients. However, it is very likely that for many of the patients reported here compliance with the restricted diet was poor. Thus, the possibility that a very strictly managed group of late-detected B₆-non-responsive patients would benefit from methionine restriction has not been excluded.

Pyridoxine administration is currently the most widely used therapy for late-detected B₆-responsive patients. A statistically significant reduction in the number of initial thromboembolic events seems to have been brought about by this treatment. Undoubtedly, this treatment, and newly recommended regimens, such as aspirin, dipyridamole, or betaine will continue to be the focus of much work in the next decade. The current data provide some insight into the magnitude of the effort required to obtain meaningful answers in such studies. Calculated over five-year intervals, the maximum likelihood for B₆-responders of having an initial thromboembolic event occurred over the interval approximately 20-25 years, when the chances were 0.038 per year. For B₆-non-responders, the maximum likelihood occurred between approximately 12.5-17.5 years, and was equal to 0.040 per year. These values correspond to about 25 years/event. Thus, even if patients were studied during these intervals of maximum risk, it would be necessary to follow 50 patients for five years each to accumulate the expectation that, had these patients not been treated, 10 detected thromboembolic events would have occurred. If patients were studied at earlier ages, before the periods of maximum risk, the numbers of patients and/or the periods of observation would have to be increased to attain the same expectation.

Significance to Biomedical Research and the Program of the Institute:

The emphasis in this project has been upon human genetic diseases due to defects in the metabolism of sulfur-containing compounds. Many of these diseases lead to mental retardation; understanding their etiology and pathophysiology may be expected to help in prevention of such retardation and of other serious manifestations. Work on inborn errors has repeatedly been shown to illuminate normal human biochemistry and enzymology, and study of the diseases in question have likewise proven useful in furthering understanding of normal human metabolism.

The most important outcome of the current survey of homocystinuric patients is to make available baseline data which should permit rational design and evaluation of the effect of therapies upon mental retardation, thromboembolic events, mortality, and dislocation of optic lenses. Such evaluations may have implications beyond their impact upon patients with severe forms of homocystinuria. Evidence continues to be reported implicating mild methionine intolerance as a major risk factor in early coronary artery disease and cerebrovascular accidents. While not all studies support this relationship, it must be taken as a serious possibility at the moment. As many as 4×10^6 U.S. citizens are likely to have mild methionine intolerance due to heterozygosity for cystathionine β -synthase deficiency. A clearer understanding of the pathogenetic relation-

ships involved, as well as preventive modes of therapy, may potentially benefit these individuals.

Purposed Course of Research:

A manuscript describing the results of this survey has been submitted to the Am. J. Human Genetics. A response has not yet been received. It is hoped that acceptance of this paper will satisfactorily complete work on this project.

Publications:

Skovby, F., and Mudd, S.H.: Genetic Diseases of Sulfur Metabolism in Humans. In Muller, A., and Krebs, B. (Eds.): Sulfur, A Unique Element: The Significance for Chemistry, for the Geo-, Bio- and Cosmosphere and Technology. Amsterdam, The Netherlands, Elsevier Scientific Publishing Co., in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00940-04 LGCB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Methionine Biosynthesis in Higher Plants

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

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TOTAL MAN-YEARS:

1.3

PROFESSIONAL:

1.3

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

Sulfate uptake by Lemma can be described by a relatively simple model: The passage of sulfate across the cell membrane into the cytoplasm where most of it is metabolized to organic compounds or transported into the vacuole without major efflux back across the cell membrane. Vacuolar sulfate slowly re-emerges to be metabolized into protein amino acids. A comprehensive picture of the metabolic consequences of regulation of sulfate uptake in response to availability of sulfur sources was obtained. As sulfate in growth medium was increased, down-regulation of high affinity sulfate uptake was more than compensated for by unregulated uptake via a "non-saturating" system. Inorganic sulfate accumulated, but formation of reduced sulfur remained constant. Some L-cystine was converted to sulfate. Presence of L-cystine in the medium down-regulated high affinity sulfate uptake and decreased the rate of sulfate organification. Net results were dose-dependent accumulation of soluble cyst(e)ine and glutathionine, but not of total sulfate and soluble methionine. L-Methionine was not metabolized to cyst(e)ine or its products. Its presence in the medium led to increased total sulfur, brought about by many fold increases in soluble methionine, S-adenosylmethionine, and S-methylmethionine sulfonium.

A survey of the capacity of Lemma paucicostata to take up organic compounds such as might be present in the natural environment of this plant identified eight discrete transport systems. Reciprocal inhibition studies defined the preferred substrates for these systems as follows: neutral L- α -amino acids, basic amino acids, purine bases, choline, ethanolamine, tyramine, urea and aldohexoses. Each of these systems takes up its preferred substrates at high rates. The neutral amino acid systems neither transports basic amino acids nor is inhibited by these compounds. The basic amino acid system does not transport neutral amino acids but is strongly inhibited by some, but not all, of these compounds. Maintenance of these active, specific, and discrete systems in Lemma suggests they permit this plant to utilize organic compounds occurring naturally in their environment.

Project Description:

During the year, the study of regulation of sulfate transport in Lemna has been completed and uptake of organic compounds is nearing completion. It was found that sulfate uptake could be described by a relatively simple model: The passage of sulfate across the cell membrane into the cytoplasm where the great majority of it is either metabolized to organic compounds or transported into the vacuole without major efflux back across the cell membrane. Vacuolar sulfate slowly re-emerges to be metabolized into, finally, protein amino acids. The model is based on (a) uptake measurements at very short times (for determination of initial rate of uptake), (b) measurement of efflux from plants labeled to isotopic equilibrium by prolonged growth in ^{35}S sulfate and (c) pulse-chase experiments (to allow determination of extent of entry of label into cytoplasm and vacuole, and the kinetics of flow of sulfate and sulfur metabolites between these compartments). The combined results argue strongly against recently reported results of other workers (based on "compartmental analysis of ^{35}S tracer kinetics") which have been taken to indicate that typical uptake measurements do not reflect transport of sulfate across the cell membrane (due primarily to a claimed large efflux of sulfate from the cell) and that regulation of transport occurs at the vacuole membrane. In the present study, efflux was shown to be small, and the initial uptake rate was just that required to account for long-term net accumulation.

Experiments examining the regulatory response of the sulfate transport mechanism in Lemna to availability of sulfur source provide for the first time in a higher plant a comprehensive picture of the metabolic consequences of this regulation. At a low sulfate concentration (20 μM), about 99% of total sulfate uptake is by a specific sulfate transport system of high affinity and the amount of sulfate taken up provides sufficient sulfur so that neither growth rate nor protein synthesis is limited. As external sulfate is increased, the high affinity system is down regulated, and this regulation appears to be brought about by the internal pool of sulfate. A linear, non-saturating uptake system (possibly diffusion-mediated) is not regulated. At 10 mM sulfate, uptake is twice that of low sulfate plants, but 95% of the uptake is by the linear system. The excess accumulates as sulfate, and reduced sulfur remains remarkably constant in the face of the accumulated sulfate, possibly as a result of close regulation of ATP sulfurylase.

Addition of cystine to medium containing 20 μM sulfate results in down regulation of high affinity sulfate uptake (brought about by cysteine or a product of its metabolism which is not sulfate) and reduction in organification of sulfate sulfur (consistent with the down regulation of ATP sulfurylase and APS sulfo-transferase by cysteine reported by others). Cystine at 14 μM provides more than 80% of the plant's sulfur, and part of the sulfate accumulated is by virtue of cyst(e)ine desulfhydration.

Addition of methionine to medium containing 20 μM sulfate results in a moderate but non-specific decrease in high affinity sulfate uptake and an increase in total tissue sulfur. Accumulation of soluble methionine, S-methylmethionine sulfonium, S-adenosylmethionine (as well as some unidentified organic compounds) accounts for the increase. Despite the fact that soluble methionine is increased some 300-fold, there is no increase in the amount of methionine incorporated into protein, nor is there metabolism of methionine sulfur to cysteine or to

sulfate. Changes in soluble methionine are correlated with reciprocal changes in the specific activity of cystathionine γ -synthase, the first committed enzyme in the methionine biosynthetic branch.

To study uptake of amino acids and other organic compounds by Lemna we have examined the uptake of a wide array of organic compounds by determining initial rates of uptake at low substrate concentration as a measure of the constant, V_{\max}/K_m (i.e., the x-intercept on an Eadie-Hofstee plot). Since this constant is analogous to the physiological concept of "clearance" we refer to it as "maximum clearance". Maximum clearance is an association constant, or a measure of the efficiency of uptake, thereby allowing comparison between compounds. Lemna clearance values varied from as high as 1900 nl/frond/min for urea down to about 4 nl/frond/min for pyruvic acid and the pentoses. Compounds which had relatively high maximum clearances were studied further to determine the properties of the systems transporting them.

Reciprocal inhibition experiments were performed using prototype compounds chosen from structurally different groups of compounds which exhibited high clearances. Independent transport systems for the uptake of L-leucine, L-arginine, adenine, choline, ethanolamine, tyramine, urea and D-glucose were identified. For each system, the product of uptake at both low and high substrate concentrations was demonstrated to be the compound under study. For example, more than 95% of the radioactivity taken up when [^{14}C]choline (88 μM) was administered could be identified in the plant as choline or metabolites of choline. This is particularly important since in this study, as in most other uptake studies, the concentration of radioactive compound was increased by adding non-radioactive carrier to a small amount of [^{14}C]compound. Under these circumstances, radioimpurities can contribute disproportionately to the measured uptake unless the added carrier contains the same impurity or competes equally well with the uptake of the radioimpurity. These experiments further showed that most of the transported compounds were rapidly integrated into the metabolism of the tissue. For example, within 2 to 3 minutes (each compound present at 0.1 μM), some 40% of the L-leucine taken up had been incorporated into protein, 87% of adenine into nucleotides, and 94% of choline into phosphorylcholine, and within about 0.5 min 70% of D-glucose (0.2 μM) into glucose 6-phosphate and fructose 6-phosphate.

The systems responsible for uptake of the amino acids were delineated by reciprocal inhibition experiments. The prototype neutral amino acid L-leucine was a good inhibitor of each of variety of neutral L- α -amino acids tested. L-Leucine inhibited also the uptake of neutral D- α -amino acids, and of proline, 2-aminoisobutyric acid, and β -alanine. In all these cases the calculated K_i values for L-leucine were similar to one another and to the K_m for L-leucine. Further, each of the compounds tested was almost as effective an inhibitor of the uptake L-[^{14}C]-leucine as of the uptake of ^{14}C in the compound itself. These observations indicate that the major transport of all of these compounds is via a single system, the "neutral amino acid system". This system had no demonstrated affinity for the basic amino acids since these compounds failed to interfere with neutral amino acid transport, nor does any appreciable portion of basic amino acid uptake occur via this system since compounds capable of inhibiting it (L-threonine, 2-aminoisobutyric acid) had little or no inhibitory effect on basic amino acid transport.

Similar experiments examining basic amino acid uptake showed that L-arginine was about as potent an inhibitor of the uptake of L-lysine or S-methylmethionine sulfonium as it was of radioactivity in L-[¹⁴C]arginine, and both L-lysine and S-methylmethionine sulfonium inhibited the uptake of L-arginine about as strongly as they inhibited the uptake of their own ¹⁴C analogues. Thus these three compounds act as though they are transported by a common system, the "basic amino acid system". The major portion of L-cystine transport also appears to be by this system, since L-arginine was an effective inhibitor of this transport. The basic system had an affinity for some neutral amino acids (L-leucine, L-methionine) since these compounds were potent inhibitors of the uptake of the basic amino acids, but little or no affinity for other neutral amino acids (L-threonine, 2-aminoisobutyric acid). However, even those neutral amino acids which have an affinity for the basic transport system are not themselves taken up via this system, since uptake of L-leucine was insensitive to inhibition by the basic amino acids.

The number and specificity of amino acid uptake systems present in higher plants has been a matter of some controversy. It appears that results of previous studies were confounded by the neutral amino acids which have an affinity for, but are not taken up by, the basic amino acid system. Thus the results of the present study clarify this previously confusing situation.

The data also provided insights into the structural specificity determining uptake by the neutral amino acid system. At the pH at which uptakes were measured (5.9) neutral amino acids possess both a positive and a negative charge. Compounds possessing a second positive charge on the side chain (the basic amino acids) were not taken up by this system, even when this positive charge was balanced by a second negative group (cystine). Compounds with a second negative group on the side chain (L-glutamic, L-aspartic acid) were taken up relatively poorly. Increase in the bulk of the carbon side chain did not correlate consistently with any trend in altered clearance. Inversion of configuration at the α -carbon atom led to variable decreases in clearance, and increasing displacement of the amino group from the α -carbon produced progressive decreases in clearance. Methylation of the amino group of 2-aminoisobutyric acid was accompanied by a marked decrease in clearance, but the imino acid L-proline had a high clearance. Absence of the α -amino group or of the carboxyl group severely impaired uptake.

The tyramine system was, in so far as investigated, specific for this aromatic amine. The aliphatic amine, methylamine, had a very low maximum clearance, and neither L-tyrosine nor L-phenylalanine interacted strongly with this system. Lemna showed comparable maximum clearances for D-glucose and each of the other aldohexoses studied, but had much lower clearances for the ketohexose fructose, or for pentoses, sucrose or glycerol. Reciprocal inhibition studies showed that D-glucose, D-galactose, and D-mannose each inhibited the uptake of the other two sugars, and that the K_m value for each was similar to its K_i values, strongly supporting the conclusion that these aldohexoses are taken up by the same system. Results provided here concerning the specificity of the aldohexose system are the most complete yet available for higher plants. The tyramine system, and the systems for adenine, choline, ethanolamine, and urea have also not previously been described in detail in higher plants. Manuscripts concerning the uptake of organic compounds by Lemna have been submitted for publication, or are in the process of being prepared.

Significance of Biomedical Research and to the Program of the Institute:

This project is part of our general program to investigate the aspartate biosynthetic pathway in higher plants. The general significance of this research has been set forth in the report on the "Pathways of methionine and threonine metabolism, and their control, in higher plants," by Dr. Giovanelli.

Proposed Course of Research:

S-Adenosylmethionine is proposed to be a major effector in the control of methionine and threonine biosynthesis in plants. It is proposed to (1) isolate and determine the regulatory properties of methionine adenosyltransferase, (2) study in vitro inhibition of the enzyme by a variety of known reversible inhibitors, (3) search for specific irreversible inhibitors of the enzyme, (4) correlate action of inhibitors with concentrations of metabolites (e.g. methionine, S-adenosylmethionine) and fluxes catalyzed by specific enzymes (e.g. cystathionine γ -synthase, threonine synthase). Prevention of S-adenosylmethionine accumulation is expected to lead to methionine over-production.

The molecular mechanism by which methionine leads to down-regulation of cystathionine γ -synthase will be studied. Several alternate means of obtaining antibodies to this enzyme are under consideration. Identification of the enzyme on two-dimensional SDS gel electrophoresis by the antibody will give evidence as to whether down-regulation involves decreases in the steady-state concentration of the enzyme or covalent modification of it which affects electrophoretic behavior. If the amount of protein is regulated the site of regulation will be determined using standard molecular biology techniques; if regulation is by covalent modification, the basis for modification will be studied.

Publications:

Datko, A.H. and Mudd, S.H.: Sulfate uptake and its regulation in Lemna paucicostata Hegelm. 6746. Plant Physiol. 75:466-473.

Datko, A.H. and Mudd, S.H.: Responses of sulfur-containing compounds in Lemna paucicostata Hegelm. 6746 to changes in availability of sulfur sources. Plant Physiol. 75:474-479.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00941-04 LGCB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biochemical Genetics and Metabolic Disease

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1

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CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The primary goal of this study has been to explore the application of new biochemical methodologies to human genetic diseases affecting the central nervous system. A concentrated effort has been made with the techniques of high resolution two-dimensional electrophoresis (2DE) autoradiography and ultra-sensitive silver staining. Continued efforts to develop more reliable staining methods has resulted in a photodevelopment silver stain which permits visualization of proteins 10 minutes after electrophoresis.

Initial studies utilizing these technics involved a disorder with known biochemical alterations, the Lesch-Nyhan syndrome. Currently, we are investigating a disease with a suspected genetic basis, familial Alzheimer's disease. Large multigenerational pedigrees have been utilized. These pedigrees serve a dual role, they permit a greater chance to establish trait specific markers than smaller families, and they also serve as a resource in the development of a human genome map based on polymorphic markers. The establishment of a human genome map based on polymorphic marker loci will be an invaluable aid in investigations of any disease which contains a genetic component. The protein polymorphisms identified in this study will complement the DNA polymorphisms which are being identified in other laboratories. We have established cell lines from the individuals in the pedigrees under investigation. These cells are permitting investigations of additional paradigms for these diseases, such as the possible role for viral genes, which have been integrated into the human genome, and the use of specific DNA probes.

A number of diseases involving the central nervous system (CNS) have been included in a survey utilizing the high resolution 2DE with silver staining to search for alterations in cerebrospinal fluid (CSF) proteins. Altered CSF protein patterns have been found in Herpes Encephalitis, Creutzfeldt-Jakob disease, Parkinson's disease, multiple sclerosis and in 40% of the schizophrenics examined.

Other Investigators:

D. Klein	Research Physiologist	LDN NICHD
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Project Description:

In an effort to extend our perception of the individual at the molecular level, we are exploring the applications of new biochemical methodologies. We have concentrated our effort on the technique of two-dimensional electrophoresis (2DE). This is a technique which permits the separation, identification and measurement of several thousand gene products which can be synthesized by any cell type. The use of such technology will allow increasingly accurate recognition, classification, understanding and treatment of pathology. There are four major areas in which high resolution 2 DE may contribute to human medical genetics: 1) the identification of primary mutations in genetic diseases; 2) the study of molecular alterations in inborn errors which may provide disease-specific and disease-associated markers; 3) observations of metabolic perturbations which will lead to a better understanding of normal metabolism and to the elucidation of metabolic patterns and pathways of diseases; and 4) the identification of protein polymorphisms and use of these polymorphisms in gene mapping.

Because genetic variants with metabolic consequences usually alter the quantity or quality of proteins, identification of alterations in protein quantity, composition, functional activity, localization, immunological determinants, or electrophoretic mobility have all contributed to our understanding of particular genetic diseases. In addition, these methods have helped reveal extensive, apparently selection-neutral, protein genetic variation. An unknown fraction of protein genetic variants confer gross structural and phenotypic variation and are the biochemical basis of phenotypic individuality. These are the genetic factors which help define us as individuals both in health and in disease.

Two-dimensional electrophoresis extends the capacity for detecting and studying the extent mechanisms and consequences of genetic variation, chiefly because it enables more than 1000 proteins to be visualized from a single cell type and can be applied with little modification to different cells and tissue fluids and their subfractions. Sensitive visualization methods, such as that achieved by the silver stains developed in this laboratory allow largely indiscriminate detection so that the high resolving power of 2DE can be fully exploited. In a continuing effort to increase the utility of silver staining, we recently developed a "photodevelopment" silver stain which permits visualization of protein and nucleic acid patterns 10 minutes after an electrophoretic separation. Discriminate detection, as with radiolabeling of phosphorylated proteins or detection with specific antibody is also useful. The large number of proteins resolved and the ready-made two-dimensional matrix make possible the systematized mapping and cross-correlation of new data, especially when 2DE is combined with the computer-assisted measurement and data reduction, systems we have participated in developing.

Detection of Protein Polymorphism

Individual variation has both genetic and environmental origins. Genetic and environmental variation are manifested grossly or only on the molecular level, but all genetic phenotypic variation is determined by molecular variation and is most precisely studied at the molecular level. The study of the subtle molecular differences will lead to the dissection and understanding of human individuality. A category of normal genetic variation is the polymorphism. Polymorphisms are normal genetic phenotypic variants found with an allelic frequency of greater than 0.02. Although usually thought of as selectively neutral, an unknown fraction produce subtle metabolic and other phenotypic effects. Protein profiling by 2DE may help uncover which do so.

Of more immediate interest is the role molecular polymorphisms have to play for linkage analysis and for constructing a map of the human genome. For Drosophila, an effective genetic map was pieced together by Morgan and his students by 1926. This map was based solely on external characteristics and omitted only the Y chromosome. However, the process of mapping the human genome has lagged because of its greater size, the long species generation time and the fact that crosses cannot be arranged for experimental purposes and mutants cannot be deliberately induced in our society. However, the availability of molecular polymorphisms, in combination with linkage analysis and the use of somatic cell genetics, has now brought this goal within reach. Molecular polymorphisms which are generally useful for assigning new loci by linkage analysis are ones whose frequency is great enough that there is a reasonable likelihood that they will be present in a family under study.

Currently, less than 50 commonly polymorphic loci are available, including allozyme polymorphisms detectable by one-dimensional electrophoresis, serological antigen loci and DNA restriction fragment length polymorphisms (RFLPs). Discovery of new polymorphisms results in the probability that some additional fractions of the genome will be covered for genetic linkage studies. Currently, approximately 26% of the human genome is covered by linkage studies at a recombination fraction of 10%. Utilization of the polymorphisms available by twodimensional electrophoresis will greatly increase the fraction covered if it can be shown that the polymorphisms being discovered are new ones. In a computer-assisted analysis of 80 individuals we have found 40 polymorphisms among 354 proteins surveyed in human lymphocytes, and fibroblasts.

In each case, the polymorphism is detected by a charge shift. In a general fashion, the extent of this shift in charge was found to vary inversely with molecular weight. Within the pH range examined, 2DE is thus capable of detecting almost all of the allozyme variants detected by one-dimensional electrophoresis because these variants generally involve charge shifts.

In addition to the shift in pI, there is often a slight shift in apparent molecular mass. This shift in SDS polyacrylamide gel mobility may be caused by altered conformation of the denatured protein in the second dimension gel and has been frequently observed for charge-substituted variants. Some recently described serum polymorphisms showed a shift in apparent molecular mass but no

shift in charge. Such variants could involve charge-neutral amino acid substitutions which result in a conformational change or which alter post-translational processing.

Gene dosage for 2DE protein polymorphisms has generally been observed when it has been looked for quantitatively. We have been able to demonstrate a gene dosage in each of the polymorphisms identified in our studies.

In the area of human population genetics, 2DE has enabled us to greatly expand the number of loci which can be assessed for variability. The over-all level of genetic variability in a population is expressed as average heterozygosity. Average heterozygosity is computed by summing the heterozygosity observed for each locus scored and dividing by the total number of loci scored. The average heterozygosity detectable by allozyme electrophoresis was 6.3% for man. We found an average heterozygosity of 2.4% for the 186 proteins surveyed in our human lymphocyte samples.

Although considerable progress has been made in the art of detecting 2DE polymorphisms, extensive family studies are still at an early stage of development.

Detection of Protein Mutation by Two-Dimensional Electrophoresis

Discovery of new protein mutations and primary protein abnormalities in genetic disease by 2DE is an exciting prospect because no other technique allows so many gene products to be surveyed in a single assay.

Some of the first clinical studies of inborn errors of metabolism involving abnormal behavioral patterns were done on the Lesch-Nyhan syndrome. The Lesch-Nyhan syndrome is an X-linked recessive trait due to a deficiency of hypoxanthineguanine phosphoribosyl transferase (HPRT) activity and results in spasticity, hyperuricemia and self-mutilation. Because HPRT activity was often undetectable and because some antibodies prepared against normal HPRT did not recognize mutant HPRTs, it was hypothesized that most of these patients were totally deficient in the protein. The mutations responsible for total absence of a protein could be mutations in a control protein or mutations at the structural locus which resulted in either a failure in transcription or early termination of translation (amber mutation). In E. coli, we successfully employed 2DE to demonstrate amber mutations in three enzymes of the galactose metabolic pathway: uridine diphosphate galactose 4-epimerase, galactokinase and galactose 1-phosphate uridyl transferase. However, in the Lesch-Nyhan syndrome, we resolved, visualized and measured HPRT protein in each of three Lesch-Nyhan patients despite the virtual absence of enzyme activity and virtual absence of immunoprecipitable HPRT. In each case, there was only a slight reduction in HPRT protein on 2DE gels.

The detection of primary mutation remains the most difficult task in the investigation of genetic diseases. The major reasons for this are as follows:

1. Two-dimensional electrophoresis probably surveys only a fraction of the total cellular proteins. Based on the apparent number of mRNA species, the number of active cellular structural genes in somatic tissues is between 10,000 and 20,000, and the number is perhaps somewhat higher in brain, for which the data ranges

from 12,000 to 70,000. If the mutation involves a minor polypeptide or one which is not visualized, it is likely to be missed. To overcome this difficulty, one may study tissues and subcellular fractions which are more likely to contain the protein whose mutation is responsible for the disease.

2. Even if the mutant protein is detectable, it may not show a charge alteration and it may be present in nearly normal concentration although its functional level is greatly reduced, as occurred in our Lesch-Nyhan study. One way of overcoming this difficulty is to study a sufficient number of subjects with a disease so that genetic heterogeneity will make likely the study of some individuals who manifest a charge mutation.

3. Charge variants discovered may be coincidental polymorphisms and must be proven to be otherwise.

4. Protein alterations not detected as charge variants are likely to be secondary alterations as described below. Such proteins can be useful as trait- or state-specific molecular markers and may give clues to pathogenesis.

Although detection of the specific mutation in a disease of unknown molecular origin is still unlikely, 2DE is far superior to any other method for this purpose. Two-dimensional electrophoresis also holds considerable promise for detecting the effects of mutagens, both in vitro and in the whole organism.

Characteristic Patterns of Polypeptide Modulation

Genetic traits and biological states may be associated with characteristic patterns of protein modulations. Some diseases which are phenocopies on a gross level may reveal trait-specific molecular markers due to secondary effects of the primary mutation.

The effects we are concerned with detecting are due to feedback control mechanisms, post-translational modification, altered protein stabilities or disrupted compartmentation. Patterns of protein modulation can be state-specific, or associated, or trait-specific or associated. The development of a correlative catalog of protein modulations in different diseases and will greatly assist in defining the specificity of protein modulations or patterns of modulations and will help dissect pathogenic and normal processes.

Patterns of state-associated protein modulations have been quantitatively characterized by exposing cells or tissues to a variety of treatments. These include heat shock, nerve growth factor, dibutyryl cAMP, calcium and thyroid hormone. Proteins have also been shown to vary with the cell cycle and after neoplastic transformation. In most cases, none of the observed modulations can be said to be state-specific because few treatments have been examined and because the identity and biology of modulation of most of the altered polypeptides is not understood.

Trait-associated protein alterations have now been identified in a number of human diseases. Merrill et al. found eleven significant quantitative alterations in which the quantitative alteration was greater than two-fold, among 400 lymphocyte protein surveys in patients with the Lesch-Nyhan syndrome. These differences

were present in the patterns of all Lesch-Nyhan patients examined and were not found in any normal individuals. Secondary protein modulations have also been found in alterations of chromosome number, as in Trisomy 21. Proteins from aneuploid individuals may also display increased or decreased quantities which are secondary to gene dosage. In this context, such alterations might also be classified as trait-associated modulations. In this way, cytoplasmic superoxide dismutase (SOD-1) which is known to map to Chromosome 21 and which shows enzyme activity proportional to the number of copies of Chromosome 21, shows a density proportional to gene dosage when examined on 2DE gels of cells mono-, di- and trisomic for Chromosome 21. It should be noted that altered copy number for one chromosome may alter expression of genes on other chromosomes. For instance, activities of several enzymes which map to chromosomes other than 21 have been shown to be elevated in Down's syndrome. Nevertheless, using this approach, we have tentatively mapped two proteins, SOD-1 and an unknown 2DE protein, to Chromosome 21.

Studies of the tissue which are primarily involved in a disease process have usually yielded extensive differences when compared to the tissue in the normal state. These differences are at times due to cell death and altered cellular composition of the tissue. Brains of patients with Huntington's disease, Joseph's disease and multiple sclerosis show extensive protein alterations but most of these are secondary to gliosis. When peripheral fibroblasts and lymphocytes have been examined in Huntington's disease, no polypeptide alterations were detected. In our survey, although more than 300 proteins were quantitatively analyzed in 13 patients and 15 controls and individuals at risk for Huntington's disease, no abnormalities were observed in the patterns examined.

Despite the difficulties noted in the paragraph above there are certain CNS diseases which have previously displayed few if any physical changes in tissues or body fluids from patients. The use of high resolution 2 DE and silver staining has revealed a number of disease specific protein alterations in CSF, from patients with multiple sclerosis, herpes simplex encephalitis, Creutzfeldt-Jakob disease and in 40% of schizophrenic patients. We had previously demonstrated that we could visualize over 300 protein in CSF and we identified 26 of these. Patients with multiple sclerosis, SSPE, and herpes simplex encephalitis all demonstrated additional immunoglobulin light chain species. We have also demonstrated that improved discrimination between MS and other neurological disorders may be achieved by quantitative assessment of the immunoglobulin light chains and quantitation of three additional protein spots (proteins 102, 39, and 15). Examination of CSF from Jakob-Creutzfeldt disease revealed two new proteins in the region of the light immunoglobulin light. These new proteins failed to stain light chain antibodies and are probably not IgG component proteins. These proteins have also be observed in experimental Kuru. Patients with herpes simplex encephalitis have demonstrated two new proteins with charges similar to albumin but with lower molecular weights. These proteins were observed in 86% of herpes encephalitis patients and 40% of schizophrenic patients.

Significance to Biomedical Research and the Program of the Institute:

Clinical studies utilizing 2DE may contribute knowledge for diagnostic markers and on polymorphism and understanding of normal disease and processes. The discovery of disease specific CSF protein alterations may also provide leads toward

the understanding of the pathophysiology of the disease process. Our observation of a specific CSF protein alteration in Herpes encephalitis and in 40% of schizophrenic patients is a "case-in-point". A viral origin of some forms of schizophrenic has been suggested by a number of investigators. The observation of similar protein alterations in Herpes encephalitis and 40% of schizophrenic patients may provide physical evidence of such an association. In the area of protein polymorphisms, 40 human polymorphisms, including 24 which may be new ones, have been described and better estimates of human protein heterozygosity have been provided. For primary mutations, 2DE has been successfully used to identify charge-shift and amber mutations. For secondary protein modulations, patterns of these have been demonstrated in a number of genetic diseases. The specificity of these alterations as markers will be established as additional diseases and metabolic alterations are studied. The prospect of correlating the information that is being accumulated is good owing to the suitability of the high resolution 2DE matrix to cataloging and the application of the method to a growing variety of questions. Many diseases of the central nervous system can be diagnosed only by their symptoms. Evidence of genetic involvement has been obtained by family studies in some of these diseases and biochemical abnormalities have been reported in a few. The techniques developed in this project will permit surveys for trait-specific and state-specific disease protein markers on a scale that would not have been possible in the past proposed course of research.

Proposed Course of Research:

We would like to continue studies of cerebrospinal fluid proteins, concentrating on the determination of the identity of the CSF proteins which are observed to be altered in CNS diseases. We would also like to extend these studies to include greater numbers of patients in each group. In our studies of familial Alzheimer's disease, we have chosen to utilize large multigenerational pedigrees. The use of such pedigrees will serve a dual role: they will permit a greater chance of establishing a definitive linkage with disease markers, and they will also serve as a resource for the development of a human genome map. Such a map will be an invaluable aid in investigations of all diseases with genetic components. We would also like to establish cell lines from these pedigrees. These cell lines will permit investigations of additional paradigms for these diseases, such as the role of viral genes which are integrated in the human genome. An effort will be made to extend the capability of the 2DE system so that small peptides can also be monitored.

Publications:

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Heydorn, W.E., Creed, G.J., Goldman, D., Kanter, D., Merrill, C.R. and Jacobowitz, D.M. Mapping and quantitation of proteins from discrete nuclei and other areas of the rat brain by two dimensional gel electrophoresis, The Journal of Neuroscience 3, 2597-2606, 1983.

Nash, W. G., Kamerow, H.N. and Merrill, C.R.: Identification of a yellow genes specific protein in Drosophila melanogaster by two dimensional gel electrophoresis. Biochemical Genetics 21: 1135-1142, 1983.

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Merrill, C.R., Harrington, M. and Alley, V.: A photodevelopment silver stain for the rapid visualization of proteins separated on polyacrylamide gels. Electrophoresis, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00942-03 LGCB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biochemical Reactions in Mammalian Cell Chemotaxis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I. G. L. Cantoni Chief, Laboratory of General and Comparative Biochemistry LGCB NIMH

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PROFESSIONAL: 1.5 OTHER: 0.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Chemotaxis by the RAW264 mouse macrophage cell line was inhibited by 3-deazaadenosine but not by 3-deazaaristeromycin. Observations of the cells by time-lapse video photography indicated that treatment of cells with 3-deazaadenosine inhibited signal processing. A search for biochemical reactions inhibited by 3-deazaadenosine but not by 3-deazaaristeromycin has revealed that only one reaction, the synthesis of a small number of proteins identified after separation by two-dimensional polyacrylamide gel electrophoresis, has the necessary inhibitor specificity for involvement in the 3-deazaadenosine-sensitive step of chemotaxis. A correlation was found between inhibition of chemotaxis and inhibition of the synthesis of same subset of proteins when other compounds were tested. These compounds also inhibited the synthesis of polyadenylated mRNA, leading us to postulate that incubation of cells with 3-deazaadenosine inhibits a methylation reaction that is required for the formation of functional mRNA coding for one or more proteins required for chemotaxis. Experiments with broken cells indicated that the inhibition of the synthesis of proteins by 3-deazaadenosine was not at the level of translation.

Experiments to identify attractant-specific proteins have been limited because chemically defined attractants for RAW264 cells have not been available. This problem has been overcome by the isolation of a stable cell hybrid from a fusion between human leukocytes and a thioguanine-resistant RAW264 cell line. The hybrid expressed functional genes for chemotaxis to N-formylmet-leu-phe, a commercially available synthetic attractant. The hybrid was similar to RAW264 cells in morphology and inhibition by 3-deazaadenosine and 3-deazaaristeromycin.

Bacterial toxins may provide another probe of reactions involved in chemotaxis. RAW264 chemotaxis was inhibited by cholera toxin and pertussis toxin. Cholera toxin did not appear to exert its inhibitory action by increasing the levels of cAMP, since treatment of RAW264 cells with other compounds which increased cAMP to comparable levels, such as forskolin and isoproterenol, did not inhibit chemotaxis.

Other Investigators:

D. Carotti	Guest Researcher	LGCB NIMH
T. M. Caryk	Chemist	LGCB NIMH
L. Harvath	Research Microbiologist	OB NCI
B. D. Meade	Research Microbiologist	DBBP BB

Project Description:

The important discovery in this laboratory that chemotaxis by a macrophage cell line is specifically inhibited by 3-deaza-AdoHcy has allowed us to assess the significance of certain biochemical reactions in macrophage chemotaxis. Our conclusion was based on the finding that RAW264 chemotaxis is inhibited by 3-deazaadenosine but not by 3-deazaaristeromycin, and a search was initiated for a biochemical reaction that also showed this inhibitor specificity.

The synthesis of phosphatidylcholine by methylation of phosphatidylethanolamine, the release of arachidonic acid when cells are incubated with EAMS (endotoxin-activated mouse serum, an attractant for mouse macrophages), methylation of the lysine and arginine residues of protein, and protein carboxymethylation were all inhibited by both 3-deazaadenosine and 3-deazaaristeromycin. From these studies we conclude that none of these reactions are required for chemotaxis by RAW264 cells.

In contrast, the synthesis of one or a small number of proteins, identified after separation by two-dimensional polyacrylamide gel electrophoresis, does show the necessary inhibitor specificity for involvement in RAW264 chemotaxis. Quantitation of 100 of the more prominent proteins on the gels by computerized densitometry showed that in cells treated with 3-deazaadenosine the synthesis of approximately 10% of the proteins was inhibited by more than 50%, whereas in cells treated with 3-deazaaristeromycin the synthesis of these proteins was not significantly inhibited. The correlation of the inhibition of a subset of proteins with the inhibition of chemotaxis was strengthened by the finding that other inhibitors of chemotaxis inhibited the synthesis of the same subset of proteins. These inhibitors are 3'-deoxyadenosine and the combination of erythro-9-(2-hydroxy-3-nonyl)-adenosin (EHNA), adenosine and homocysteine. A common feature of the inhibitors of chemotaxis described above is that they all can inhibit the synthesis of functional mRNA. In this regard, we have also found that inhibitors of protein synthesis and translation, such as cycloheximide, puromycin and actinomycin D, inhibit chemotaxis.

We have proposed as a working hypothesis that treatment of RAW264 cells with 3-deazaadenosine, 3'-deoxyadenosine, and the combination of EHNA, adenosine and homocysteine inhibit the synthesis of functional mRNA coding for one or more chemotactic proteins. In support of this hypothesis, we have found that 3-deazaadenosine is a more potent inhibitor of polyadenylated mRNA than 3-deazaaristeromycin and that AdoHcy and 3-deazaAdoHcy do not inhibit in vitro translation.

Time-lapse video cinematography shows that motility and EAMS-induced morphological changes are similar in 3-deazaadenosine-treated and control cells. These observations suggest that in cells treated with 3-deazaadenosine, signal processing after attractant binding to the chemoreceptor is inhibited.

Additional studies to examine directly the effects of 3-deazaadenosine on attractant binding or to investigate the steps in signal transduction have been hindered by the lack of chemically defined attractants. The attractants described for RAW264 cells, EAMS and LDCF (lymphocyte-derived chemotactic factor), are both complex biological fluids. On the other hand, human monocytes and neutrophils are known to exhibit chemotaxis to FMLP (N-formylmet-leu-phe), a commercially available synthetic attractant. For these reasons hybrids cells were isolated from fusions between human leukocytes and thioguanine-resistant RAW264 cells, and many of the hybrids exhibited chemotaxis to FMLP. Initial characterization of one of these hybrids shows that chemotaxis to both FMLP and EAMS is inhibited by 3-deazaadenosine but not by 3-deazaaristeromycin, indicating that data obtained with these inhibitors for RAW264 may also be applicable to the hybrid cells. This hybrid cell line has been cultured for more than 6 months without loss of chemotaxis to FMLP demonstrating that a stable cell line has been obtained. This cell line should allow us to identify and characterize attractant-specific reactions.

A study relating to the human FMLP receptor has been carried out in collaboration with Dr. L. Harvath on the effects of oxidized FMLP on chemotaxis and the generation of superoxide anion by human neutrophils and monocytes. This laboratory's principal contribution has been the preparation and analytical determination of FMLP sulfoxide and sulfone. These studies have shown that human monocytes exhibit chemotaxis for both FMLP sulfoxide and sulfone, whereas human neutrophils do not exhibit chemotaxis to either of the oxidized peptides. In contrast, both human neutrophils and monocytes migrate to FMLP and both cell types generate superoxide anion when stimulated with FMLP, FMLP sulfoxide or FMLP sulfone. These data suggest that the FMLP receptor complex or chemotaxis transduction mechanism is different in human neutrophils and monocytes.

Reports in the literature and results from this laboratory suggest that an inter-relationship between cAMP and AdoHcy metabolism may exist. During the course of studies designed to examine the effects of increased levels of cAMP upon the activity of AdoHcy hydrolase, it was found that chemotaxis by RAW264 cells was not inhibited when intracellular cAMP was increased by treatment of the cells with forskolin or isoproterenol. However, the chemotaxis of cells treated with cholera toxin, which raised intracellular cAMP to levels that were comparable to those achieved by forskolin and isoproterenol, was inhibited. These data show that increased levels of cAMP per se do not inhibit chemotaxis and suggest that either the binding of cholera toxin to the cell surface or the ADP-ribosylation reaction catalyzed by cholera toxin may be involved in the inhibition of chemotaxis. In this regard, RAW264 chemotaxis is also inhibited by pertussis toxin, another bacterial protein with ADP-ribosylating activity.

Significance of Biological Research to the Program of the Institute:

Several reports have shown that stress-induced neuropeptides modulate immunological activities and that leukocytes have receptors for beta-endorphin. Chemotaxis is an important component of the immunological response, and it has been shown that human monocytes exhibit chemotaxis to met-enkephalin and beta-endorphin. Injection of beta-endorphin into the rat cerebral ventricle results in the immigration of macrophage-like cells, indicating that chemotaxis to beta-endorphin

can occur in vivo. Identification of the steps involved in chemotaxis would provide a basis for the development of strategies to counteract stress-induced immunological dysfunction.

Mammalian cell chemotaxis is also important in the development of the nervous system, inflammation and wound healing, and chemotaxis is a behavioral response at the cellular level. Studies of bacterial chemotaxis from the laboratories of Koshland and Adler have shown that bacteria have "memory" and adapt to their environment, and progress has been made in explaining these concepts in molecular terms. The mammalian cell line model for chemotaxis that we have developed provides a mammalian system to test concepts developed from bacterial chemotaxis and to study the biochemical reactions involved in signal transmission.

Proposed Course of Research:

Future work will be directed toward verification of the hypothesis that 3-deaza-AdoHcy specifically inhibits the synthesis of functional mRNA coding for one or more chemotactic proteins, and toward the identification of biochemical reactions important in chemotaxis. These problems will be approached by a combination of biochemical and genetic techniques.

Publications:

Aksmit, R.R. and Backlund, P.S. Jr.: Chemotaxis and methylation in a macrophage cell line. Surv. Immunol. Res. 2: 150-154, 1983.

Harvath, L. and Aksmit, R.R.: Oxidized N-formylmethionyl-leucyl-phenylalanine: Effect on the activation of human monocyte and neutrophil chemotaxis and superoxide production. J. Immunol., in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00943-03 LGCB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pathways of Methionine and Threonine Metabolism and Their Control in Higher Plants

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

J. Giovannelli Research Chemist LGCB NIMH

Others:

S. H. Mudd Chief, Section on Alkaloid Biosynthesis LGCB NIMH

A. H. Datko Biologist LGCB NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of General and Comparative Biochemistry

SECTION

Section on Alkaloid Biosynthesis

INSTITUTE AND LOCATION

NIMH, ADAMHA, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

1.2

PROFESSIONAL:

1.2

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Efforts were concentrated on preparing manuscripts on the large body of research findings that have accumulated in past years. The experimental findings summarized below were directed at elaboration and clarification of data required for these manuscripts.

It has now been shown by direct amino acid analyses that the concentration of soluble threonine in Lemna is increased 25-fold by growth with supplemental threonine, and the concentration of soluble isoleucine is increased 70-fold by growth with supplemental isoleucine. The finding that neither down-regulation nor feedback inhibition of threonine synthase was detected in the face of these large increases in threonine and isoleucine provides convincing evidence that the expected feedback control of threonine synthase is not an important component in the regulation of threonine synthesis in Lemna. Increasing the concentration of threonine (a precursor of isoleucine) in Lemna did not appreciably affect the concentration of isoleucine, while increasing the concentration of isoleucine (70 fold) increased the concentration of threonine approximately 8-fold above that of control plants. These two observations provide the first indication of in vivo regulation of isoleucine biosynthesis in Lemna.

No currently available method is suitable for assay of [¹⁴C]methionine in the non-protein fraction of tissues extensively labeled with ¹⁴C. An assay was developed that is based on the purification of radioactive methionine by successive chromatography of its sulfoxide and carboxymethylsulfonium derivatives. The method can be applied also to assay of ³⁵S and ³H in methionine, and has the advantage of providing, for the first time, a measure of radioactivity in each of the three moieties of methionine.

Project Description:

Efforts were concentrated on preparing manuscripts on the large body of research findings that have accumulated in past years. Consequently, no new projects were initiated, and the experimental results outlined below were directed at elaboration and clarification of data required for manuscripts:

(a) While threonine synthase would be expected to be an important site for feedback regulation of threonine synthesis in Lemna, previous studies have failed to detect such regulation. For example, no down-regulation of threonine synthase was detected in Lemna grown in medium supplemented with threonine and/or isoleucine at concentrations close to the maxima which could be tolerated, and which would be expected to cause large increases in the concentrations of these amino acids in the plants. We have now shown by direct amino acid analyses that the concentration of soluble threonine in Lemna is increased some 25-fold by growth with supplemental threonine, while the concentration of soluble isoleucine is increased approx. 70-fold by growth with supplemental isoleucine. The finding that neither down-regulation nor feedback inhibition of threonine synthase was detected in the face of these large increases in threonine and isoleucine provides convincing evidence that the expected feedback control of threonine synthase is not an important component in the regulation of threonine synthesis in Lemna.

(b) Increasing the concentration of threonine (a precursor of isoleucine) in Lemna did not appreciably affect the concentration of isoleucine, while increasing (70-fold) the concentration of isoleucine increased the concentration of threonine approx. 8-fold above that of control plants. These combined observations provide the first evidence for in vivo regulation of isoleucine biosynthesis in Lemna.

(c) No satisfactory method is available for determination of non-protein [^{14}C]methionine in tissues that are extensively labeled with ^{14}C . This determination is especially difficult in plants since they are characterized by a wide variety of non-protein unusual secondary products. A procedure was developed for determination of nonprotein [^{14}C]methionine in the presence of at least a 2000-fold excess of ^{14}C in undefined compounds. The method was based on successive purification by paper chromatography of the derivatives methionine sulfoxide and methioninecarboxymethylsulfonium salt. An advantage of the procedure is that it also provides, for the first time, a measure of radioactivity in each of the three moieties of methionine. This measurement is obtained by degradation of methioninecarboxymethylsulfonium salt to homoserine (4-carbon moiety) and methylthioacetate, which is then oxidized to carbon dioxide (methyl moiety) and inorganic sulfate (sulfur moiety). Attempts to prepare [^3H]methioninecarboxymethylsulfonium salt by reaction of methionine with iodo- ^3H acetate revealed a facile labilization of ^3H on the (carboxy)-methylene carbon of methioninecarboxymethylsulfonium salt. Our studies suggest that this lability requires the combined presence of adjacent sulfonium and carboxyl groups, since no labilization was observed of ^3H on the methylene carbon of carboxymethylcysteine, nor on the methyl group of methioninecarboxymethylsulfonium salt. It is proposed that labilization of ^3H in methioninecarboxymethylsulfonium salt proceeds by dissociation of ^3H to yield a sulfur

ylid, which would be expected to be stabilized through delocalization of carbanion electrons through the carboxyl group, and into the vacant 3d orbital of sulfur.

Significance to Biomedical Research and the Program of the Institute:

The primary goal of this project is to elucidate the pathways for methionine and threonine metabolism, and their control, in higher plants, using Lemna as an experimental system. Methionine and threonine biosynthesis are closely related in plants, the two pathways branching at the common intermediate O-phosphohomoserine. There are now a number of indications that regulation of the two biosynthetic branches may also be interrelated. Our research on each of these two amino acids therefore continues to be directed along parallel and complementary lines. This project is significant to the research goals of the Institute since methionine and threonine are among the four most commonly limiting essential amino acids in the human diet. Deficiency of these amino acids (especially during early life) in protein-calorie malnutrition may be accompanied by irreversible retardation in mental development. Plant proteins provide the source of these two amino acids almost entirely, either directly by ingestion of plant material, or indirectly through an animal intermediate. Many of the plant foods most used by man are deficient in one or both of the amino acids, methionine and threonine. An understanding of the patterns of control of the biosynthesis and metabolism of methionine and threonine will provide a rational basis for maximizing the production of these essential dietary components.

Proposed Course of Research:

A major priority is to complete the publication of our backlog of research findings.

Proposals (1) to (3) listed below are focused on localizing the major sites at which regulation of the aspartate family of amino acids occurs in plants, and on defining the mechanisms of regulation at these sites. Clarification of these problems is of critical importance in accomplishing our long range goal of providing a rational basis for improvement of the nutritional content of the aspartate family of amino acids in plants.

- (1) The extent of in vivo regulation of threonine and isoleucine biosynthesis in Lemna will be quantitated.
- (2) The major regulatory site(s) for threonine biosynthesis will be determined. This promises to be an intriguing problem since our studies indicate that regulation does not occur by the expected feedback regulation of threonine synthase. The possibility will be examined that threonine controls its own synthesis by feedback regulation at threonine-sensitive aspartokinase, and channeling of the products of this enzyme specifically into the threonine biosynthetic branch.

(3) Evidence that the allosteric stimulation of threonine synthase by S-adenosylmethionine (AdoMet) plays a key role in regulating fluxes into the methionine and threonine biosynthetic branches in plants is based mainly on in vitro studies. We propose to examine directly the in vivo significance of this proposed regulatory pattern by determining the in vivo fluxes of 4-carbon units into the methionine and threonine biosynthetic branches in Lemna containing varying concentrations of AdoMet. The in vivo concentrations of AdoMet in Lemna will be increased or decreased relative to that of control plants by growth on supplemental methionine or inhibitors of methionine adenosyltransferase, respectively.

Although the regulatory patterns for synthesis of the aspartate family of amino acids remain to be elucidated, current knowledge suggests that aspartokinase and threonine synthase are two sites at which synthesis of these amino acids is limited. Subject to the resources, time and manpower available, we plan to initiate studies aimed at increasing the synthesis of methionine and threonine by genetic engineering of the genes for aspartokinase and threonine synthase. Initial studies will concentrate on preparing highly purified preparations of these enzymes for production of antibodies. The availability of purified threonine synthase will also allow us to extend our studies on the regulatory properties of the Lemna enzyme, especially those concerning the allosteric stimulation by AdoMet and potent inhibitions by AMP and phosphate. Furthermore the availability of plant aspartokinases for the first time in highly purified forms would provide excellent opportunities for examining the catalytic and regulatory properties of these enzymes.

Publications:

Giovanelli, J., Veluthambi, K., Thompson, G.A., Mudd, S.H., and Datko, A.H.: Threonine synthase of Lemna paucicostata Hegelm. 6747. Plant Physiol. In press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00981-19 LNB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Detection and interpretation of mechanical changes in the nervous system

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Ichiji Tasaki, Chief, Laboratory of Neurobiology, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Neurobiology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

3

PROFESSIONAL:

2

OTHER:

1

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Since the discovery of the phenomenon of swelling in invertebrate nerve fiber in 1980, our effort has been directed toward the following two goals: (1) to improve our mechanoelectric transducers and expand our investigation to include other excitable cells and tissues, and (2) to clarify the nature of the phenomenon of swelling on a physicochemical basis. Last year, we found that the frog dorsal root ganglion swells when invaded by an afferent volley of impulses. We found also that there is a large mechanical movement in the frog spinal cord following stimulation of the dorsal roots.

During the year just past, we found complex movements of the isolated retinas of the squid, crab, lobster and bullfrog. Since invertebrate eyes contain a limited number of photosensitive cells, our analyses of the newly discovered phenomenon was straightforward. We came to the conclusion that the movement of water molecules associated with release and uptake of Ca-ions play a crucial role in photoelectric transduction. A study of mechanical movements of the bullfrog retina is in progress.

Quite recently, we found rapid mechanical movements of the bullfrog sympathetic ganglion produced by ortho- and anti-dromic volley of impulses. In addition to the swelling associated with action potential production in the preganglionic fibers and in the ganglion cells, we could record, from deeply curarized ganglia, mechanical changes associated with the excitatory postsynaptic potential. We believe that studies of these mechanical changes in synapses yield important information as to the mechanism of synaptic transmission.

Project Description:

Others involved in this project are Toshio Nakaye, Visiting Fellow, NIMH, LNB and Paul M. Byrne, Biomedical Engineering Technician, NIMH, LNB.

Objectives:

Elucidation of the molecular basis of excitation processes in the nervous system by detecting and analyzing mechanical changes in nerve cells, fibers and synapses.

Methods Employed:

A variety of excitable systems of the crab, lobster, squid and bullfrog were employed to study the involvement of the phenomenon of swelling in excitation, conduction and synaptic transmission. Mechanoelectric transducers employed include (1) piezoceramic bender (purchased from Gulton Industries, Inc.); (2) small aluminum levers used in conjunction with a Fotonic sensor (Mechanical Technology, Inc.); and (3) polyvinylidene film (Kureha Chemical Co.).

Major Findings:

(1) Demonstration of Swelling and Shrinkage in the Visual Systems of the Squid, Lobster and Bullfrog.

We found that the squid retina responds to brief light pulses with rapid mechanical movements. The movement observed is diphasic: following the delivery of a light pulse, the retina first swells and then shrinks. Since these movements take place on the same time-scale as the production of electroretinogram, we conclude that these movements play a crucial role in generation of electric responses of the retina. (See Science, 1984.)

We have extensively examined the mechanical changes in the lobster eye generated by light stimulation. We have established that the spectral sensitivity curve determined by taking mechanical responses as an index coincides with the known absorption spectrum of the retinal visual pigment. Next, we have shown that the time-course of the mechanical response reflects that of the impedance change in the eye. The manuscript describing these and other new findings has been submitted for publication.

(2) Analyses of Synaptic Transmission by the Use of Mechanoelectric Transducer.

We have shown that electric stimulation of the preganglionic nerve fibers produces rapid mechanical changes in the lumbar sympathetic ganglion of the bullfrog. The mechanical changes consist of three distinct components: (i) swelling of the preganglionic fiber terminals, (ii) swelling of the ganglion cell somas associated with the production of action potential and (iii) relatively slow swelling associated with the generation of the excitatory postsynaptic potential. The last component of the mechanical response was

detected after curarization of the ganglion. Since the synaptic transmitter involved in this ganglion has been identified as acetylcholine, we infer that the action of acetylcholine is to enhance the membrane conductance by causing swelling of the ganglion cells. A manuscript describing these new findings will be submitted for publication in the near future.

Scientific Significance and Relevance to Public Mental Health:

The phenomenon of swelling of nerve fibers and cells is directly related to changes in the interaction of membrane and cytoskeletal proteins with small ions and water molecules. There is little doubt that such changes in the degree and the type of interaction are at the base of the normal process of excitation and inhibition in the nervous system. We believe, therefore, that experimental studies along the aforementioned line led us to a better understanding of the normal function, as well as abnormal behavior, of the nervous system.

Proposed Course:

We have just started our investigation into the contractile processes in chemical synapses. We are planning to continue to examine these mechanical changes in relation to other physicochemical and electrophysiological events that take place in other synapses. A study of visual processes in the frog retina is in progress.

Publications:

Tasaki, I. and Byrne, P. M. Swelling of frog dorsal root ganglion and spinal cord produced by afferent volley of impulses. *Br. Res.* 272: 360-363, 1983.

Tasaki, I. and Byrne, P. M. Mechanical changes in the amphibian spinal cord produced by afferent volleys of nerve impulses. *Br. Res.* (in press).

Metuzals, J., Clapin, D. F., and Tasaki, I. The axolemma-ectoplasm complex of squid giant axon. In: Structure and Function in Excitable Cells. Dr. Chang, I. Tasaki, W. Adelman, and R. Leuchtag (eds.), Plenum Publishing Corp., pp. 53-73, 1983.

Tasaki, I. and Iwasa, K. Axolemma-ectoplasm complex and mechanical responses of the axon membrane. In: Structure and Function in Excitable Cells. Dr. Chang, I. Tasaki, W. Adelman, and R. Leuchtag (Eds.), Plenum Publishing Corp., pp. 307-319, 1983.

Tasaki, I., Nakaye, T., and Byrne, P. M. Swelling of axon membrane during excitation. (Presented at the XXIX International Union of Physiological Sciences.) Adelaide, So. Australia and Sydney, Australia, 8/25-9/3-83, in press.

Tasaki, I. and Nakaye, T. Rapid mechanical responses of the dark-adapted squid retina to light pulses. *Science* 223: 411-413.

Tasaki, I. and Nakaye, T. Rapid mechanical changes in the nervous system during excitation. (Presented at the Deiriken Conference, Okazaki, Japan., Dec. 1983.) In press.

Iwasa, K. Osmotic properties of the squid giant axon and their modifications. *Cell. Mol. Biol.* 3:151-161, 1983.

Iwasa, K. Anion-dependent swelling of crab nerve fibers during potassium and veratridine depolarization. *Physiol. Chem. Phys.* 14:503-512, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00983-06 LNB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biochemical studies on the mechanism of nerve excitation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Jesse Baumgold, Research Chemist, Laboratory of Neurobiology, NIMH

COOPERATING UNITS (if any)

Laboratory of Neurogenetics, BPB, NIMH

LAB/BRANCH

Laboratory of Neurobiology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

2

PROFESSIONAL:

1

OTHER:

1

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Further studies on the development of sodium channel proteins from rat brain were carried out. By using neurotoxins specific for different parts of the sodium channel protein, we found that the sodium channel protein undergoes conformational changes during development. In early stages of development, we found two ^{125}I -scorpion toxin sites per ^3H -saxitoxin site, whereas at later stages of development, the stoichiometry of these two sites had changed to two ^3H -saxitoxin sites per ^{125}I -scorpion toxin site. Despite this changing stoichiometry, both forms of this protein behaved identically on lectin affinity columns, ion exchange columns and on SDS-polyacrylamide gel electrophoresis, suggesting that no biochemical differences were detectable.

In another project, muscarinic receptor protein from rat brain was partially purified using preparative isoelectric focusing, lectin affinity chromatography and gel permeation chromatography. This affinity labeled protein binds to, and can be specifically eluted from wheat germ affinity columns and has a molecular weight of 80,000 daltons and an isoelectric point of 5.9.

Project Description:

Objectives:

The project involving the biochemical characterization of sodium channel proteins described in last year's report has been completed (see Major Findings).

A project in collaboration with Dr. E. Gershon, Laboratory of Neurogenetics, Biological Psychiatry Branch, has been undertaken. Dr. Gershon and collaborators have previously found that skin fibroblasts from patients with depressive or manic-depressive disorders express a higher level of muscarinic receptor protein than those of controls. Since these psychiatric disorders are frequently inherited, it was of interest to undertake a program to study abnormalities in the genes for muscarinic receptors from depressed patients versus controls.

The general approach used in this project has been to purify muscarinic receptor protein from rat brain, microsequence a portion of the peptide chain, and from the amino acid sequence, make an oligonucleotide probe.

Methods Employed:

(1) Affinity labeling of the muscarinic receptor with ^3H -propylbenzylcholine mustard.

(2) Standard methods of purification of membrane proteins including solubilization of these proteins with detergents, chromatography on lectin-affinity columns and on molecular sieving columns.

(3) Electrophoretic methods of protein purification, including preparative isoelectric focusing and preparative polyacrylamide gel electrophoresis.

Major Findings:

(1) Sodium Channel Project.

By using two neurotoxins (^3H -saxitoxin and ^{125}I -scorpion toxin) that bind to different parts of the sodium channel protein, we found that the stoichiometric ratio of these two sites varies dramatically during ontogenetic development of rat brains. By post-natal day 7, we found 2 scorpion toxin sites per saxitoxin site, whereas in adult rats, this ratio had dropped to 0.5. This finding suggests that the protein undergoes major conformational changes during development. We also found that the immature form of this protein has the same molecular weight, the same net charge and the same lectin specificity as the mature form of this protein, suggesting that this conformational change is difficult to detect and identify biochemically.

(2) Muscarinic Receptor Project.

The affinity-labeled muscarinic receptor from rat brain was solubilized with detergents and substantially purified, using preparative isoelectric

focusing, lectin affinity chromatography and preparative SDS polyacrylamide gel electrophoresis. The resulting pure protein has a molecular weight of 80,000 and an isoelectric point of around 5.9.

Based on pharmacological binding experiments, two subtypes of muscarinic receptors have been proposed: M_1 receptors and M_2 receptors.

Frontal cortex has been shown to contain predominantly M_1 receptors, whereas the pons medulla and the cerebellum contain predominantly M_2 receptors. When we compared affinity-labeled receptors proteins from these parts of the brain, we found that, regardless of the brain areas examined, they all had molecular weights of 80,000 and isoelectric points of 5.9 and 6.1. This suggests that the pharmacological differentiation into subtypes is due to conformational changes in the protein and not due to separate proteins.

Genetically inbred strains of mice have been very useful in behavioral research. We compared the levels of muscarinic receptors in four of such inbred strains that exhibited different degrees of aggression or passivity, and found that all four strains had similar levels of muscarinic receptors.

Scientific Significance and Relevance to Public Mental Health:

Clinical depression and manic depression are known to be hereditary diseases suggesting genetic and, therefore, biochemical etiologies. The recent findings implicating muscarinic receptors in these diseases represents a possible direction for further investigation into the biochemistry of these complex diseases.

Proposed Course:

We are planning to complete work on the purification of the muscarinic receptor protein, to have the purified protein microsequenced, and to make an oligonucleotide probe based on that sequence. This probe will be useful in screening clinical samples for possible restriction fragment polymorphism associated with manic depression.

Publications:

Baumgold, J., Zimmerman, I., Bambrick, L. Appearance of ^3H -saxitoxin binding sites in developing rat brain. *Devel. Brain Res.*, 9, 405-7, 1983.

Baumgold, J. Changes in the ratio of two separate toxin binding sites on the sodium channel protein during rat brain development. *Devel. Brain Res.*, 1984, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 01036-12 LNB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Protein and Nerve Function by Modulator-Sites on...

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Audrey L. Stone, Research Chemist, Laboratory of Neurobiology, NIMH

COOPERATING UNITS (if any)

University of Maryland, Department of Chemistry,
Laboratory of Cerebral Metabolism, NIMH,
Laboratory of Genetics, NCI.

LAB/BRANCH

Laboratory of Neurobiology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

1.7

PROFESSIONAL:

1.1

OTHER:

.6

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The heparin and heparin sulfate class of glycosaminoglycans (GAG) have extraordinary variation in the secondary features of the disaccharide repeating units implicated in the modulation of some enzymes, proteins and cell membranes. We characterized oligosaccharide (oligos) from these GAG and related their structural properties to their multiple biological functions. The molecular basis of the reactions between heparin and heparin sulfates and these proteins was investigated by uv circular dichroism (CD) spectroscopy of the proteins and then complexes with oligosaccharide modulators. The two functional domains of heparin (contained within an octadecasaccharide, octadecas) that differentially activate antithrombin were investigated by low uv CD spectroscopy of their oligos along with model compounds. The disaccharide sequence between the two functional regions was elucidated, enabling us to propose a sequence for the anticoagulant octadecas. The metachromatic reaction of methylene blue with this oligos partially supports our proposed structure, which consists of alternating regions of higher and lower anionic density that are flanked by highly sulfated disaccharides. We studied the conformation of oligos using our technique of Induced CD spectroscopy. Computer-calculated theoretical extrinsic CD spectra corresponded with the experimental spectra. Oligos were isolated and characterized for experiments on neuronal development along with media conditioned over bovine embryonic kidney and human fibroblast cells. The effects of these agents on the morphological differentiation of adult chromaffin cells of the bovine adrenal (AMC), and various embryonic neuronal cells was studied. Although AMC from rat have been reported to exhibit neurite outgrowth following treatment with these agents, we found no such effect on AMC from either adult bovine or calf.

Project Description

Other investigators engaged on this project are Mariam M. Dohadwalla, Visiting Fellow, Howard DeVoe, Assoc. Prof. of Chemistry, Univ. of Maryland, and Ross E. Martenson, Res. Chemist, ICI, MML, Dr. L. Coon, NCI.

The project concerns the structure and function of the heparin and heparin sulfate class of complex carbohydrates and their possible relation to neuronal function and development. It is speculated that their multifunctional reactivity derives from the chemical properties of various unique oligosaccharides (oligos).

Objectives:

Subproject 1: To determine the molecular basis of the Modulation of the Biological Reactions of Proteins by Heparins and Heparin Sulfates; to analyze the conformation of oligopeptide fragments of myelin basic protein (MBP).
Subproject 2: To determine the Structure-Function Relations of Modulator Sites in Heparin-type Complex Carbohydrates. Subproject 3: To study the Role of Heparin Sulfate and HS-Proteoglycans in Neuronal Development and Function, to characterize factors in conditioned media and to define their possible role in development of CNS and in the mental retardation associated with the Sanfilippo mucopolysaccharidoses.

Methods Employed:

Standard biochemical techniques are used to isolate proteins, complex carbohydrates and oligos generated by various acid cleavage. Assays of biological activity are spectrophotometric or by dot radioassay. Conformational aspects of the proteins and oligos are determined by near and far circular dichroism (CD) spectroscopy (modified Cary 60 and Jasco 500 spectropolarimeters) and by dot technique of extrinsic CD spectroscopy. (A computer program established with Prof. L. Holtz predicts the extrinsic CD and molar ellipticity spectra of methylene blue bound to arrays of anionic sites on oligos.) The CD of oligopeptides of MBP is measured in the conformation-creating solvent trichloroethane, (TCE) and the fractions of alpha helix, beta-sheet, beta-turn and non-ordered coil conformations determined by "best fit" analysis.

Dissociated cells are obtained by microscopic dissociation of embryonic rat and mouse cerebellum and cerebral cortex, and by retrograde enzyme perfusion of adult bovine adrenal glands. Using standard techniques, an SH^+ -transferrin of cerebellar cells from the 13-day rat embryo (with Dr. Harold Coon) is maintained. Media cultured over bovine embryonic kidney (BEK) and human skin fibroblast (F) cells are collected for use as experimental substratum. Extensive morphological examination of the cells and selected electrophysiological measurements (under batch-culture) are made.

Major Findings:

Subproject 1: We obtain excellent agreement between "best-fit" and experiment far CD spectrum of the 96-105 polypeptide of MBP when we utilize

our polypeptide models. The CD spectra of 36-16% varies as a function of percent age of IFE in the solvent. Best-fit analysis shows that this fragment has relatively weak tendency to form α -helical structure while having the potential of up to 40 percent at the expense of the coil form. Theoretical estimate of α -helical potential from amino acid sequence (by R. Martenson) was 25 percent. Subproject 2: We now propose a sequence of the anticoagulant octadecad of heparin that contains the second modulator region of the reducing side of the major binding site. This sequence is partially supported by the metachromatic reaction of MB bound to the various oligos. Furthermore, among numerous predictions of extrinsic CD spectra of bound MB there is exceptionally good correspondence between the experimental spectrum and the prediction based on the repeat sequence of the decad. Subproject 3: Dissociated cell cultures from the adult or calf bovine adrenal gland (AMC) are stable up to two weeks. They do not exhibit the neurite outgrowth, typical of sympathetic neurons in the presence of nerve-growth-factor (NGF) when cultured in the absence presence of substratum derived from media conditioned over B2K or F, in contrast with corresponding cell cultures of the rat. Addition of GAG fractions with or without conditioned media substratum also fails to evoke such a morphological change. However, the GAG (unfractionated, oligos and larger fractions) and other polyanions permeabilize the AMC to small molecules such as erythrosin B. Development of partially differentiated neuronal cells are seen at a low frequency when DBW-1A transformants are plated at a low density over conditioned media, but this cell model remains only potentially useful. Primary cultures of dissociated cells from embryonic cerebellum appear to be stable up to 10-12 days and may serve well as a model.

Scientific Significance and Relevance to Public Mental Health:

Over a number of years our investigations have elucidated special attributes of GAG. The reactions of heparin in promoting anticoagulation are prototypical. Our ideas regarding the role of these oligos modulators in mental health research relates their special attributes to the complex process of cell migration in the developing CNS. We believe that heparin sulfates are involved in defining the routes between the neuron and target cell. The variability in oligos sequences appears to have an 'orderliness'. Short segments of relatively low anionic density are flanked by a different number of highly sulfated disaccharides. (See Stone, A. L.: 'Far Ultraviolet Circular Dichroism and Uronic Acid Components of Deca-, Dodeca-, Tetradeca-, and Octadeca-saccharide Heparin Fractions that Bind to and Activate Antithrombin.' Submitted to Arch. Biochem. Biophys.) The decad size may be functionally important in the macromolecule. Computer analysis of theoretical extrinsic CD spectra of metachromatic ligands develops a probe that selects for the presence of particular oligos sequences that might occur in a number of macromolecules. (Results presented: Annual Meeting Society for Complex Carbohydrates, October, 1983, p. 24, Stone, A. L., Winter, W. L., and DeLoe, R. G.: 'Theoretical extrinsic circular dichroism of metachromatic ligands bound to oligosaccharide segments of heparin (H) and other glycosaminoglycans (GAG).')

AMC cells are used widely as a neuronal model. They are also used in surgical, longterm replacement of degenerated dopaminergic neurons. Ready

permeabilization of AMC by heparin sulfate and other GAG has direct import in studies of intracellular modulation where permeability barriers to small molecules is rate limiting. (A manuscript entitled, "Permeabilization of adult bovine chromaffin cells by glycosaminoglycans" by Mariam M. Dohadwala and Audrey L. Stone, in preparation.)

Studies of the conformations of MBP oligopeptides enable a better understanding of the compact structure of MBP, the putative antigen of multiple sclerosis, in the myelin sheath of the CNS.

Proposed Course:

Subprojects 1, 2, and 3: will be continued.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
 NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01031-16 LNC

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Conversion of Phenylalanine to Tyrosine

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI	Seymour Kaufman	Chief	LNC NIMH
	Masayoshi Iwaki	Visiting Fellow	LNC NIMH
	Desirazu Narasimha Rao	Staff Fellow	LNC NIMH
	Michael Davis	Staff Fellow	LNC NIMH

COOPERATING UNITS (if any)

Dr. S. Benkovic, Department of Chemistry, Penn State University, University Park

LAB/BRANCH

Laboratory of Neurochemistry

SECTION

INSTITUTE AND LOCATION

ADAMHA, NIMH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

3.7

PROFESSIONAL:

2.7

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Spectral measurements have proved that activation of phenylalanine hydroxylase changes its conformation. A 4 α -carbinolamine tetrahydropterin has been shown to be an intermediate in the hydroxylation reaction. Phenylalanine hydroxylase stimulator protein has been shown to be a 4 α -carbinolamine dehydratase. Glucagon stimulates phenylalanine hydroxylase activity in the whole rat.

Major Findings:

Previous results from our laboratory, as well as from others, have suggested that phenylalanine hydroxylase can exist in at least two different conformations, a low activity and a high activity state. Activators of the enzyme, such as its substrate, phenylalanine, and the phospholipid, lysolecithin, are believed to convert the enzyme from the low to the high activity state, whereas the pterin cofactor, tetrahydrobiopterin (BH_4), is believed to do the opposite. We have now obtained the first direct evidence in support of this two state model for the hydroxylase. Incubation of the enzyme with either phenylalanine or lysolecithin increased the fluorescence at 360 nm; this increase paralleled the increase in hydroxylase activity. By contrast, BH_4 , which can deactivate the enzyme, quenched the fluorescence. These results prove that the conformation of the enzyme in the presence of activators is different from that in the presence of a deactivator.

We have also obtained evidence against the prevailing view that the conformation of phenylalanine hydroxylase in the presence of phenylalanine and lysolecithin is the same. Studies of the effect of these two activators on the enzyme's ultraviolet circular dichroism spectrum have shown that the physical state of the enzyme in the presence of phenylalanine is quite distinct from the state in the presence of lysolecithin.

Our earlier work had led to the identification of an intermediate in the hydroxylation reaction, which we had postulated was a tetrahydropterin 4 α -carbinolamine. We had also purified to homogeneity a protein from rat liver, phenylalanine hydroxylase stimulating protein (PHS), which we showed was capable of converting the intermediate to the final pterin product, quinonoid-dihydropterin. In studies carried out in collaboration with Dr. S. Benkovic, we have proven the validity of: (a) our proposed structure of the pterin intermediate; and (b) the postulated role of PHS; i.e., we have proved that PHS is a 4 α -carbinolamine dehydratase.

Our previous work had demonstrated that rat liver phenylalanine hydroxylase can be activated in vivo by the pancreatic hormone, glucagon. This activation was shown to be due to enhanced phosphorylation of the hydroxylase by the action of a cyclic-AMP-dependent protein kinase. In those experiments, the hormone was administered to rats, the livers were excised, and the hydroxylase activity was measured in vitro. We have now extended this work by showing that phenylalanine hydroxylase activity is higher in situ in glucagon-treated rats. Using a whole animal perfusion technique, in which rats are given a constant perfusion of phenylalanine until a steady-state concentration of blood phenylalanine is reached, we have shown that the injection of glucagon produces a prompt decrease in the concentration of phenylalanine in blood. In addition, we have shown in similar experiments in which radioactive phenylalanine was perfused, that glucagon administration leads to an increase in the specific radioactivity of tyrosine. These results prove for the first time that glucagon activates phenylalanine hydroxylase in situ. The extent of activation in situ, however, is significantly less than it is in vitro.

Significance to Biomedical Research and Proposed Course of Project:

The changes in fluorescence and ultraviolet circular dichroism spectra of phenylalanine hydroxylase in the presence of activators provide convincing evidence in favor of a two-state model for the enzyme. We plan to carry out further studies on the physical properties of the activated enzyme to try to understand the molecular basis for the enhanced catalytic activity. A deeper understanding of this process could lead to new ways to regulate the enzyme's activity in vivo.

Another aspect of the regulatory properties of phenylalanine hydroxylase was established with our demonstration that the enzyme is in an activated state in glucagon-treated rats and that the enhanced activity can be detected in situ. Our calculation of the extent of activation in situ indicated that it was less than half as great as the maximum activation that can be achieved when the enzyme is phosphorylated in vitro by cyclic-AMP-dependent protein kinase. We showed that this apparent discrepancy could be explained by differences in temperatures between the in vivo and in vitro assay conditions, being 37°C for the former and 25°C for the latter condition. These results led to the novel and unexpected finding that at physiological temperatures, rat liver hydroxylase is already in a partially activated state. Using the physical techniques discussed above, we plan to determine whether the conformation change that occurs at elevated temperatures resembles that seen when the enzyme is activated by phenylalanine and by lysolecithin.

Publications:

1. Phillips, R.S., and Kaufman, S.: Ligand effects on the phosphorylation state of hepatic phenylalanine hydroxylase. J. Biol. Chem. 259, 2474-2479, 1984.
2. Phillips, R.S., Parniak, M.A., and Kaufman, S.: Spectroscopic investigation of ligand interaction with hepatic phenylalanine hydroxylase: Evidence for a conformational change associated with activation. Biochemistry, 1984. (In press)
3. Phillips, R.S., Parniak, M.A., and Kaufman, S.: The interaction of aromatic amino acids with rat liver phenylalanine hydroxylase. J. Biol. Chem. 259, 271-277, 1984.
4. Lazarus, R.A., Benkovic, S.J., and Kaufman, S.: Phenylalanine Hydroxylase stimulator protein in a 4 α -carbinolamine dehydratase. J. Biol. Chem. 258, 10960-10962, 1983.
5. Phillips, R.S., and Kaufman, S.: On the nature of the spontaneous activation of hepatic phenylalanine hydroxylase. Transactions of the N.Y. Acad. Sciences. 41, 87-95, 1983.

6. Parniak, M.A., and Kaufman, S.: Irreversible inactivation of rat liver phenylalanine hydroxylase by reaction with (6S)-L-erythro-tetrahydrobiopterin. In: Chem. and Biol. of Pteridines. J.A. Blair, (ed.), W. de Gruyter, Berlin. 345-349, 1983.
7. Dhondt, J.L., Kapatos, G., Parniak, M.A., Wilgus, H., and Kaufman, S.: Biopterin and phenylalanine metabolism during early liver regeneration. In: Chem. and Biol. of Pteridines. J.A. Blair, (ed.), W. de Gruyter, Berlin. p. 851-856, 1983.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 01032-16 LNC

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biosynthesis of catecholamines

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI	Seymour Kaufman	Chief	LNC
	Kenneth Davis	Staff Fellow	LNC NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Neurochemistry

SECTION

INSTITUTE AND LOCATION

ADAMHA, NIMH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

1.2

PROFESSIONAL:

1.2

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects
 (b) Human tissues
 (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Tyrosine hydroxylase catalyzes the rate-limiting step in the biosynthesis of the neurotransmitters dopamine and norepinephrine. We are purifying the enzyme from bovine striatal tissue.

Project Description:

The objective of this research project is the detailed description of the hydroxylation reactions that are involved in the biosynthesis of the neurotransmitters, dopamine and norepinephrine. Recently, we have been focusing on attempts to purify tyrosine hydroxylase from brain in order to further clarify the molecular mechanism of activation of this enzyme by phosphorylation.

Major Findings:

We are trying to devise a high-yield, large-scale purification of the enzyme from bovine striatal tissue.

Significance to Biomedical Research Proposed Course of Project:

Tyrosine hydroxylase catalyzes the rate-limiting step in the reaction sequence leading from tyrosine to dopamine and norepinephrine. Any factor that can alter the activity of this enzyme can change tissue levels of dopamine and norepinephrine. One of those factors is activation of the enzyme by phosphorylation. We plan to continue to try to purify the enzyme from brain tissue.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01034-16 LNC

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Biochemical Basis of Skeletal Muscle Hypertrophy

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI Seymour Kaufman
Michael Bissell

Chief
Staff Fellow

LNC NIMH
LNC NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Neurochemistry

SECTION

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ADAMHA, NIMH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

1.7

PROFESSIONAL:

1.2

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The object of this work is the elucidation of the biochemical mechanism of compensatory skeletal muscle hypertrophy. These studies will give greater insight into the physiological regulation of normal and pathologic skeletal muscle metabolism and into the role of the nervous system in this regulation. They may also prove to be generalizable to the study of adaptation of other organs and tissues to increased physiological demand (e.g., the nervous system).

We have found that Indomethacin inhibits the stretch-induced stimulation of protein synthesis in chick embryo muscle. This result suggests that prostaglandins may be involved in mediating the stretch response.

Project Description:

Passive mechanical stretching of skeletal muscle leads to hypertrophy of the tissue. Using a technique developed in this laboratory for stretching monolayers of chick embryo myotubes *in vitro*, we have shown that the response of skeletal myotubes to passive stretch includes increased amino acid uptake (as measured by uptake of amino-isobutyric acid, AIB), increased incorporation of amino acids into, and accumulation of, total cellular protein. These increases are inhibited by ouabain after a 30-minute lag period and by tetrodotoxin. Stretch is also associated with an early increase in the V_{max} of the membrane Na/K-dependent ATPase, as measured by Rubidium-86 uptake. These stretch effects take place in serum-free medium and are mimicked by the addition of serum to unstretched cultures.

We have also carried out studies on a whole animal model of skeletal muscle hypertrophy. This *in vivo* tenotomy model involves the surgical section of the Achilles tendon of the gastrocnemius muscle of one limb while a "sham" operation is carried out on the other limb. Following the operation, the weight-bearing load is redistributed from the gastrocnemius to the two smaller synergist muscles, the soleus and plantaris, which rapidly hypertrophy. Using this system, we have seen significant increases in wet weight of the soleus and plantaris, as early as four to six hours post-tenotomy.

Major Findings:

We have found that the anti-inflammatory drug Indomethacin inhibits the stretch-induced stimulation of protein synthesis. The increase in amino acid transport that is elicited by stretch is also partially inhibited by the drug. This we believe to be a secondary effect, since blocking protein synthesis with cycloheximide also abolishes the stretch-induced increase in amino acid transport.

Significance to Biomedical Research Proposed Course of Project:

Our finding that Indomethacin, an inhibitor of cyclo-oxygenase can inhibit stretch-induced protein synthesis, may imply that the formation of prostaglandin plays some part in the stretch response. Arachidonic acid may be released from lipids in the stretched cell membrane and act as part of the chemical stimulus to hypertrophy. This idea is congruent with our earlier finding that phosphatidyl inositol turnover is increased in tenotomized rat muscle undergoing hypertrophy.

We would like to extend these studies to the area of neuronal growth and determine whether neurones respond to stretch in the same way as do muscle cells. Due to a cut in the laboratory's resources, however, we will be unable to continue this project.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 01035-16 LNC

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Process of Lysogeny

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI	Howard Nash	Medical Officer (Research)	LNC NIMH
	Nancy Craig	Staff Fellow	LNC NIMH
	Paul Kitts	Visiting Fellow	LNC NIMH
	Evelyne Richet	Visiting Fellow	LNC NIMH
	Robert Weisberg	Chief, Section on Microbial Genetics	LMG NICHD
	Gerald Zon	Director, Molecular Pharmacology Br.	MPB, FDA
	H. Weisbach	Director of Research	Roche Institute
	J. Griffith	Professor	UNC, Chapel Hill

COOPERATING UNITS (if any)

Laboratory of Molecular Genetics, NICHD; NIAMDD; Division of Biochemistry & Biophysics, Center for Drugs & Biologics, FDA; Roche Institute of Molecular Biology, Nutley, NJ; Lineberger Cancer Research Center, University of North Carolina, Chapel Hill, NC

LAB/BRANCH

Laboratory of Neurochemistry

SECTION

INSTITUTE AND LOCATION

ADAMHA, NIMH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

4.5

PROFESSIONAL:

3.5

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have shown that a recombination protein directly affects gene expression. The E. coli integration host factor alters the action of RNA polymerase, apparently by affecting its tendency to terminate elongation of RNA. This protein, together with a viral recombination protein, organize DNA into a wrapped structure prior to recombination. Studies of the topology of DNA recombinant products indicate the handedness of this wrapping is unique and resembles that found in chromatin. Alterations of the chemistry of recombining sites has been achieved; the semisynthetic DNA substrates for recombination are specifically blocked in breakage and reunion.

Project Description:

There are two outcomes of infection of the bacterium *E. coli* by the bacterial virus lambda. In the lytic pathway, the virus makes many copies of itself and ultimately ruptures the host cell, releasing the viral progeny. In the life cycle that leads to lysogeny, most viral genes are repressed and the viral DNA is inserted into the host chromosome. Our research is concerned with the mechanism of the genetic recombination that leads to the integration of viral DNA. Our earlier work established an in vitro system for the biochemical analysis of this reaction and we subsequently identified and purified the proteins and cofactors that take part in this recombination. Our current studies seek to learn how these proteins direct the recombination and how they affect host metabolism.

Of the two proteins required for integrative recombination, one is encoded by the *E. coli* host, the other by the virus. Genetic and biochemical analyses of the host protein, called integration host factor (IHF), indicate that it is a heterodimer of two small polypeptides. The behavior of mutants in the genes for these subunits have indicated that IHF regulates genes expression in *E. coli*. In collaboration with Dr. H. Weisbach of the Roche Institute of Molecular Biology, we have undertaken a biochemical analysis of one such regulated system. We have examined the production of phage protein, the product of the lambda *cII* gene, in a purified system that transcribes DNA into message and translates message into protein. We find that, as in vivo, addition of IHF stimulates this expression 3-5 fold. IHF does not stimulate gene expression from other operons on the same piece of DNA and *cII* expression is not stimulated by another *E. coli* protein whose size and charge are similar to IHF. Dissection of the purified system shows that the stimulation of gene expression by IHF occurs during transcription. Expression of several genes located downstream of *cII* are also stimulated but expression of the gene lying upstream of *cII* is not affected by IHF. We therefore conclude that this recombination protein also has the capacity to affect RNA polymerase, probably modulating its tendency to terminate transcription.

Integrative recombination takes place only at special sequences in DNA. Earlier work from this laboratory has led to the conclusion that the special recombination site carried by the virus, *attP*, is organized into a complex structure during recombination. This structure presumably forms by wrapping of *attP* DNA around a core of protein created by interactions between IHF and *Int*, the viral protein required for integrative recombination. Similar structures have been observed for DNA associated with the histone proteins in chromatin and for DNA interacting with complex enzymes like DNA gyrase. In the former case, the DNA is wrapped around the histone core in a left-handed manner and in the latter case the DNA is wrapped in a right-handed manner. We have asked if there is a unique mode of wrapping of DNA in the structure formed at *attP* during recombination and, if so, what the handedness of this structure is. We had earlier shown that as a result of wrapping of *attP*, circular DNA substrates recombine to form knotted circular products. There is a fixed relationship between chirality of the wrapped *attP* site and the chirality of recombinant knot produced from it. Therefore, in collaboration with J. Griffith of the University of North Carolina, we have determined the handedness of the recombinant knots by purifying these

Project Description:

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structures from *in vitro* recombination reaction mixtures, coating them with a DNA binding protein, and examining them in the electron microscope. We find that all the knots made by recombination have the same handedness; the handedness is of the type that implies a wrapping of attP identical to that observed in chromatin. By establishing that the att site is wrapped in a constant manner, we have provided strong evidence against the possibility that wrapping is accidental and conversely have substantiated our suggestion that wrapping reflects an important step in recombination. Moreover, the finding of a constant wrap goes a long way in explaining the observation that supercoiling of DNA benefits recombination. This is because the strain introduced by supercoiling favors formation of structures wrapped with the handedness that we have deduced for attP.

One of the key steps in recombination is synapsis, the juxtaposition of segments of DNA prior to strand exchange. It is at this step that recognition of nucleic acid homology probably occurs and therefore understanding synaptic structures is central to the understanding of many kinds of recombination. In an attempt to stabilize synaptic structures, we are developing novel substrates for recombination. These substrates have nucleotides with unusual chemistry positioned precisely at the locus of the recombination crossover. We seek those substituents that make DNA a poor substrate for strand exchange but permit normal initiation of recombination. Our first attempts have focused on derivatives in which the phosphodiester backbone is altered. By a combination of chemical and enzymatic synthesis we have constructed variants of attB, the *E. coli* site of lambda integration, in which certain phosphodiester bonds are replaced by the corresponding phosphorothioate derivative. When this substitution occurs at the position normally involved in strand exchange, recombination is depressed about 10 fold. On the other hand phosphorothioate substitution at several other positions within attB is without deleterious effect. This specificity implies that thiophosphate substitution specifically blocks strand exchange. Encouraged by this result, we have examined such reaction mixtures for complexes involving pairs of DNA helices. Our preliminary results are encouraging; when Int-h, a variant recombination protein with enhanced recombination activity, is incubated with these substrates, unusual recombinant products are found. The kinetics of appearance of these products, their dependence on denaturants, and their resistance to challenge by salt all indicate that they arise from a moderately stable synaptic intermediate.

Significance to Biomedical Research and Proposed Course of Project:

Recombination between specific sequences on DNA is a key strategy for differentiation in several biological systems. In higher organisms, it has become clear that the entire immune response is intimately regulated by site-specific recombination. In bacteria, many more examples of gene regulation that depend on site-specific recombination are known. Our work on determining the mechanism of one particular site-specific recombination is both a basic contribution to a little understood class of DNA transformations and a tool for understanding these reactions in more complicated organisms.

We want to delve further into the mechanism by which IHF affects gene expression. We plan to study the interaction of this protein with nusA and rho, two important regulatory proteins that affect termination of transcription. In addition, we will investigate the nature of the RNA produced in the presence and absence of IHF. We plan to examine possible alterations in the amount, start point, terminus, and secondary structure of such RNA.

We will pursue our strategy of creating semisynthetic recombining sites. We will characterize any synaptic intermediates that we can detect as to life time, requirements for formation, and components of the complex. We plan to construct new semisynthetic sites, incorporating analogs which should probe the nature of DNA-DNA interaction in these complexes.

Publications:

1. Pollock, T. J., and Nash, H. A.: Knotting of DNA caused by a genetic rearrangement: Evidence for a nucleosome-like structure in site-specific recombination of bacteriophage lambda. J. Mol. Biol., 170, 1-18, 1983.
2. Nash, H. A., and Pollock, T. J.: Site-specific recombination of bacteriophage lambda: The change in topological linking number associated with exchange of DNA strands. J. Mol. Biol., 170, 19-38, 1983.
3. Craig, N. L., and Nash, H. A.: The mechanism of phage lambda site-specific recombination: Collision versus sliding in att site juxtaposition. In: Mechanisms of DNA Replication and Recombination, (N. Cozzarelli (ed.)). UCLA symposia on Molecular and Cellular Biology, 10, 617-636, 1983.
4. Craig, N. L., and Nash, H. A.: The mechanism of phage site-specific recombination: site-specific braking of DNA by Int topoisomerase. Cell, 35, 795-803, 1983.
5. Lange-Gustafson, B. J., and Nash, H. A.: Purification and properties of Int-h, a variant protein involved in site-specific recombination of bacteriophage lambda. J. Biol. Chem. (In press)
6. Nash, H. A., Kitts, P. A. and Richet, E. R.: Lambda integrative recombination: supercoiling, synopsis and strand exchange. Cold Spring Harbor Symposium on Quantitative Biology. (In press)

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 01037-16 LNC

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Role of the Cell Membrane in Cellular Organization: A Molecular Study

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

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	Richard Youle	Research Biochemist	LNC NIMH
	Jon Marsh	Staff Fellow	LNC NIMH
	Thomas Hudson	Staff Fellow	LNC NIMH
	Daniel A. Valleria	Assistant Professor	Univ. of Minnesota
	J. H. Kersey	Professor of Pediatrics	Univ. of Minnesota

COOPERATING UNITS (if any)

Minnesota Bone Marrow Transplantation Group, University of Minnesota,
Minneapolis, Minnesota

LAB/BRANCH

Laboratory of Neurochemistry

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Biophysical Chemistry

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ADAMHA, NIMH, Bethesda, Maryland 20205

TOTAL MAN-YEARS

6.0

PROFESSIONAL:

4.0

OTHER:

2.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The general aim of this project is to determine the chemical interactions which occur at the surfaces of cells which affect cellular differentiation and organization. Specifically we have studied one type of intereaction, plasma membrane receptor mediated entry of proteins into the cell cytosol. These studies have been done by developing techniques to construct artificial protein conjugates containing the active fragment of a toxin and another receptor specific binding protein. Such artificial protein conjugates have value as a new class of pharmacologic reagents. Monoclonal antibody ricin conjugates or immunotoxins directed against human T cells effectively deplete these cells from donor bone marrow permitting bone marrow transplants apparently free from moderate to severe forms of graft versus host disease. This will provide a new treatment for leukemia, aplastic anemia and autoimmune diseases such as multiple sclerosis, Guillain Barre Syndrome, systemic lupus erythematosus and perhaps other diseases of the immune system such as acquired immunodeficiency syndrome. In addition, these reagents are useful for enzyme replacement therapy and in organ transplantation.

Project Description:

The general aim of this project is to determine the chemical interactions which occur at the surfaces of cells which affect cellular differentiation and organization. The major specific aim of the program is to understand how protein toxins after binding to membrane receptors are able to cross the plasma membrane and enter the cytosol compartment. These mechanisms are utilized by toxins and viruses and probably have an unknown physiologic counterpart.

A wide variety of proteins are capable of entering cells by receptor-mediated transport processes. Having gained entry these proteins are directed to specific cellular compartments where they exert either a physiological or pathological function.

In general it appears that only a discrete portion of these proteins contain the receptor binding activity which is involved prior to the entry process while another portion of the protein performs the intracellular function. Therefore, it is possible to split and reassemble two such proteins with a new combination of receptor entry specificity and intracellular function. Such proteins we call artificial hybrid proteins, and previous reports from this laboratory have suggested that such hybrids should have utility both as probes of entry processes and as a new class of pharmacologic reagents with tailor made receptor and therefore cell type specificity.

Major Findings:

1. A mixture of anti-T cell monoclonal antibody-ricin conjugates developed in this laboratory has been used to treat human donor marrow during bone marrow transplantation in order to eliminate graft-versus-host-disease. Early results are highly encouraging. Nine transplants have been performed at the University of Minnesota by substituting this new regimen for the standard methotrexate treatment of the recipient. Age and matching status of the recipients are such that a 50% incidence of moderate to severe graft-versus-host-disease (GVHD) would have been expected. So far no cases have materialized. Two cases of mild GVHD (cutaneous involvement only) have appeared and were easily controlled with steroids.
2. A large comparative study of a variety of antibody-toxin conjugates on various cell types indicates that the entry efficiency of our most effective conjugates is decreased 30-50 fold over the parent toxins when entry is normalized per thousand occupied receptors.
3. A kinetic study on the entry of diphtheria toxin (DT) into the cytosol compartment of cells reveals that entry is a quantal phenomenon. In a cell population exposed to DT the toxin enters cells via endocytotic vesicles at a uniform rate. However, entry to the cytosol compartment (where the toxin kills) appears to be a random event of rapid duration and involves many molecules of toxin. The most likely explanation is that the toxin contains a mechanism for opening a large pore or lysing the endocytotic vesicle.

Significance to Biomedical Research and Proposed Course:

1. The apparent reduction in fatal and morbid cases of graft-versus-host-disease following immunotoxin treatment of donor marrow prior to bone marrow transplant will increase the utility of this procedure and should extend its use to life threatening autoimmune diseases. A randomized clinical trial has been started to provide better data and investigation of optimal dosage of the various antibody conjugates is planned.
2. The low entry efficiency of our first generation of antibody-toxin conjugates indicates that we are not fully utilizing the toxin's inherent entry mechanisms. New approaches are being tried based on new insights into the entry mechanism.
3. The apparent discovery of how toxins cross the plasma membrane in phenomenological terms, "lysing or opening up the vesicle" is highly important in several respects. Because the mechanism is highly specific the toxin probably is utilizing a physiologic system. If this is the case, the physiologic function is unknown and this should be a fruitful area of investigation. Since the phenomenology appears settled the biochemistry should be made easier. These insights should help us to build more efficient conjugates which could be used in vivo to eliminate unwanted cells.

Publications:

1. Neville, D. M., Jr., Youle, R. J., Kersey, J. H., and Vallera, D. A.: Monoclonal antibody-Ricin Conjugates for the Treatment of Graft Versus Host Disease. Present and Future Prospects. In: Langman R. and Delbecco, R. (eds.). The Armand Hammer Cancer Symposium, Monoclonal Antibodies and Cancer. Academic Press. 107-115, 1983.
2. Vallera, D. A., Youle, R. J., Neville, D. M., Jr., Soderling, C. B., and Kersey, J. H.: Bone Marrow Transplantation Across Major Histocompatibility Barriers in Mice. VI. Anti-T Cell Monoclonal Antibody-Toxin Conjugates as Reagents for Experimental GVHD Prophylaxis are not Selectively Reactive with Murine Stem Cells. Transplantation, 36, 73-79, 1983.
3. Vallera, D. A., Ash, R. C., Zanjani, E. D., Kersey, J. H., LeBien, T. W., Beverly, P. C. L., Neville, D. M., Jr., and Youle, R. J.: Anti-T-Cell Reagents for Human Bone Marrow Transplantation: Ricin Linked to Three Monoclonal Antibodies. Science. 222, 512-515, 1983.
4. Vallera, D. A., Kersey, J. H., Quinonas, R. R., Zanjani, E. D., Soderling, C. C. B., Azemore, S. M., LeBien, T. W., Beverly, P. C. L., Ash, R. C., Neville, D. M., Jr., and Youle, R. J.: Antibody-ricin Conjugates: Purgative Reagents for Murine and Human Allogeneic Bone Marrow Transplantation. In: Recent Advances in Bone Marrow Transplantation. Gale, R. P. (ed.). New Series, Vol. 7, A. R. Liss, Inc., New York. p. 1-14, 1983.

5. Filipovich, A. H., Vallera, D. A., Youle, R. J., Quinonas, R. R., Neville, D. M., Jr., and Kersey, J. H.: Ex-vivo Treatment of Donor Bone Marrow with Anti-T Cell Immunotoxins for the Prevention of Graft-Versus-Host-Disease. Lancet, 469-472, 1984.
6. Strong, R. C., Youle, R. J., and Vallera, D. A.: Elimination of Clonogenic T Leukemic Cells from Human Bone Marrow Using Anti-p65 Immunotoxins. Cancer Res. 1984 (In press).
7. Esworthy, R. S. and Neville, D. M., Jr.: A Comparative Study of Ricin and Diphtheria Toxin-Antibody-Conjugate Kinetics of Protein Synthesis Inactivation. J. Biol. Chem. 1984 (In press).
8. Hudson, T. H. and Neville, D. M., Jr.: Quantal Entry of Diphtheria Toxin to the Cytosol. Nature. 1984 (In press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01038 16 LNC

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Phenylketonuria and Other Diseases Caused by Defects in Biopterin-Dependent Enzymes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI	Seymour Kaufman	Chief	LNC NIMH
	Sheldon Milstien	Research Chemist	LNC NIMH
	George Hoganson	Professor	Univ. of Wisconsin
	Stanley Berlow	Director, Metabolic Program	Univ. of Wisconsin
	Roderick McInnes	Professor	Hosp. for Sick Children

COOPERATING UNITS (if any)

Waisman Center on Mental Retardation and Human Development,
University of Wisconsin, Madison; Department of Genetics and Pediatrics, Hospital for
Sick Children, Ontario, Canada

LAB/BRANCH

Laboratory of Neurochemistry

SECTION

INSTITUTE AND LOCATION

ADAMHA, NIMH, Bethesda, Maryland 20205

TOTAL MAN-YEARS

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Patients with phenylketonuria (PKU) due to a defect in tetrahydrobiopterin (BH₄) synthesis who are unresponsive to pterin therapy can be treated with at least partial success with neurotransmitter replacement therapy, i.e., with Dopa and 5-hydroxytryptophan.

Project Description:

In 1975, cases of hyperphenylalaninemia were reported in which neurological disorders persist despite dietary control of phenylalanine blood levels. Subsequently, variant forms of phenylketonuria (PKU) or hyperphenylalaninemia were described by our laboratory in which the defect in the phenylalanine hydroxylase system is not in phenylalanine hydroxylase, itself, as it is in classic PKU, but rather in dihydropteridine reductase or in an enzyme involved in the biosynthesis of tetrahydrobiopterin (BH₄). Dihydropteridine reductase functions to maintain BH₄ in its functional tetrahydro form while BH₄ is an essential coenzyme. Both of these variants are therefore characterized by a marked deficiency of BH₄. Since, as previous work in this laboratory had shown, this pterin is an essential coenzyme not only for phenylalanine hydroxylase, but also for tyrosine and tryptophan hydroxylases, patients lacking BH₄ suffer from defects in the synthesis of the neurotransmitters, dopamine, norepinephrine, epinephrine and serotonin in both the peripheral and central nervous systems, as well as from an impaired ability to hydroxylate phenylalanine in the liver. Indeed, to our knowledge, these patients are the only population presently available whose neurological dysfunctions can unequivocally be attributed to a genetic defect in biogenic monoamine synthesis which does not appear to involve irreversible cell loss. These patients might therefore be considered as models for other nondegenerative neurological diseases, the etiology of which is believed to involve aberrations in biogenic monoamine metabolism.

Current therapy for these variant forms of hyperphenylalaninemia consists of restriction of phenylalanine intake and administration of the hydroxylated amino acid precursors of catecholamines and serotonin, 3,4-dihydroxyphenylalanine (DOPA) and 5-hydroxytryptophan, respectively, in conjunction with inhibition of peripheral aromatic amino acid decarboxylation with carbidopa. Although administration of BH₄ to these patients, especially to those with a defect in BH₄ biosynthesis, might also appear to be a reasonable therapy, the reports that this pterin does not readily enter the brain from the periphery made it seem unlikely that this treatment would prevent the neurological damage that characterizes these diseases.

Major Findings:

We have previously suggested, and demonstrated the effectiveness of, two different types of therapy for variant forms of PKU caused by defects in tetrahydrobiopterin (BH₄) synthesis or metabolism, namely, treatment with L-Dopa and 5-hydroxytryptophan and treatment with a tetrahydropterin. These different treatments, however, had never been compared in detail in a single patient. We have now carried out such a comparison and have found that Dopa and 5-hydroxytryptophan are partially effective in improving the patients neurological development and, in general, correcting the patient's severe deficiency of monoamines and their metabolites. By contrast, administration of 6-methyltetrahydropterin was ineffective in either improving the patients neurological function or in increasing the deficiency of monoamines, especially in the cerebral spinal fluid (CSF). These results consolidate our previous indications from patient-to-patient comparisons that there is a correlation between the ability of a treatment to correct the monoamine deficits in these patients and the ability to improve neurological function.

Significance to Proposed Course:

Our results lead to the following important conclusion: patients with defective BH_4 synthesis represent a heterogeneous group; in those patients who are responsive to pterin therapy, this therapy will likely be more effective than neurotransmitter replacement therapy; in those patients who are completely unresponsive to pterin therapy, neurotransmitter replacement therapy can still be partially effective. We plan to continue to investigate ways to improve pterin therapy for this condition.

Publications:

1. Kaufman, S., Kapatos, G., Rizzo, W.B., Schulman, J.D., Tamarkin, L., and Van Loon, G.R.: Tetrahydropterin therapy for hyperphenylalaninemia caused by defective synthesis of tetrahydrobiopterin. Annals of Neurology. 14, 308-315, 1983.
2. Kaufman, S.: Phenylketonuria and its variants. Advances in Human Genetics. 13, 217-297, 1983.
3. McInnes, Roderick, R., Kaufman, S., Warsh, J.J., Van Loon, G.R., Milstien, S., Kapatos, G., Soldin, S., Walsh, P., MacGregor, D., and Hanley, W.B.: Biopterin Synthesis Defect: Treatment with L-Dopa and 5-hydroxytryptophan compared with therapy with a tetrahydropterin. J. Clin. Invest. 73, 458-469, 1984.
4. Hoganson, G., Berlow, S., Kaufman, S., Milstien, S., Schuett, V., Matalon, R., Naylor, E., and Seifert, W.: Biopterin synthesis defects: Problems in diagnosis. Pediatrics, 1984. (In press)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01039-16 LNC

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pteridine Biosynthesis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI Seymour Kaufman Chief
Sheldon Milstien Research Chemist

LNC NIMH
LNC NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Neurochemistry

SECTION

INSTITUTE AND LOCATION

ADAMHA, NIMH, Bethesda, Maryland 20205

TOTAL MAN-YEARS

0.7

PROFESSIONAL:

0.7

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Tetrahydrobiopterin (BH₄) biosynthesis has been shown to proceed through tetrahydro intermediates. Sepiapterin reductase catalyzes the last reaction in BH₄ biosynthesis.

Project Description:

Tetrahydrobiopterin (BH_4) is the coenzyme required for the hydroxylation of phenylalanine, tyrosine and tryptophan. Children who have a genetic defect in BH_4 biosynthesis have phenylketonuria due to their inability to metabolize phenylalanine and severe neurological problems as a result of a lack of those neurotransmitters which are produced from the hydroxylated amino acids. The BH_4 biosynthetic pathway has not yet been completely elucidated. The genetic defect(s) in such children has not yet been identified, except in one case of a child who is missing the first enzyme in the pathway.

Major Findings:

In the past year, several major breakthroughs on this project have occurred in this laboratory. Previous results from several groups, as well as this laboratory, had suggested that sepiapterin, a dihydropterin, was an intermediate in BH_4 biosynthesis. For this reason, it had been postulated that the biosynthesis proceeded through pterin intermediates that were all at the dihydro level of reduction, with the last two steps being reduction of sepiapterin to dihydrobiopterin (BH_2), catalyzed by sepiapterin reductase and reduction of BH_2 to BH_4 catalyzed by dihydrofolate reductase. Both the lack of effect of potent dihydrofolate reductase inhibitors on BH_4 biosynthesis as well as the inability of BH_2 to trap radioactivity from radioactive precursors, suggested that this proposed pathway was incorrect.

However, specific inhibitors of sepiapterin reductase were very effective in inhibiting *de novo* BH_4 synthesis. This result suggested that sepiapterin reductase, an enzyme whose only previously known role was the reduction of sepiapterin to BH_2 , was catalyzing another reaction in BH_4 biosynthesis. A likely candidate that would serve as a substrate for this reaction was tetrahydro-sepiapterin, the fully reduced analogue of sepiapterin. Tetrahydro-sepiapterin was prepared and found to be directly reduced to BH_4 by sepiapterin reductase at a rate consistent with its postulated role as an intermediate. Furthermore, since it possesses some very similar properties to sepiapterin, it is clear that the early experiments that showed that sepiapterin was an intermediate were not correct because of this similarity.

Significance to Biomedical Research and Proposed Course Project:

A simple purification scheme for sepiapterin reductase has been developed. This should allow us to separate and identify the remaining unknown reactions in BH_4 biosynthesis. Antibodies to sepiapterin reductase will be prepared with the goal of obtaining a cDNA probe to screen genetic carriers of BH_4 deficiency.

Publications:

1. Milstien, S., and Kaufman, S.: Tetrahydrosepiapterin is an intermediate in tetrahydrobiopterin biosynthesis. Biochem. Biophys. Res. Comm. 115, 888-893, 1983.
2. Kapatos, G., and Kaufman, S.: Inhibition of pterin biosynthesis in the adrenergic neuroblastoma NIE115 by tetrahydrobiopterin and folate. Chem. & Biol. of Pteridines. 171-175, 1983.
3. Milstien, S., and Kaufman, S.: The regulation of biopterin biosynthesis in the rat. Chem. & Biol. of Pteridines, 753-757, 1983.
4. Milstien, S., and Kaufman, S.: Dihydrofolate reductase catalyzes the reduction of 7,8-dihydrobiopterin in liver and brain. In: Biochemical and Clinical Aspects of Pteridines, (Eds.) H. C. Curtius, W. Pfeiderer, H. Wachter, Walter de Gruyter & Co., Berlin, 1983, pp. 132-137.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 MH 01081-14 LNP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cerebral Control of Voluntary Movement

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	E. V. Evarts	Chief	LNP, NIMH
Others:	M. Kimura	Visiting Fellow	LNP, NIMH
	J. Rajkowski	Visiting Fellow	LNP, NIMH
	S. Pullman	Medical Staff Fellow	LNP, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Neurophysiology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

4.00

PROFESSIONAL

4.00

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project utilizes single neuron recording and operant conditioning techniques in behaving monkeys to study brain mechanisms underlying voluntary movement. Monkeys are trained to make movements of a handle whose position controls a visual display, and stimuli are delivered via the handle by means of an electronically controlled torque motor in order to determine how sensory feedback is processed. A major focus of work in this project has been neural activity in putamen, which is an input stage for a group of interconnected subcortical neuronal aggregates collectively referred to as the basal ganglia. Putamen receives inputs from the cerebral cortex and thalamus, is reciprocally connected with the substantia nigra pars compacta, and projects to the globus pallidus, a basal ganglia output stage. Microelectrode recordings in monkeys have shown that some putamen neurons have tonic spontaneous discharge in absence of movement; the more numerous putamen neurons that are phasically related to voluntary movement do not exhibit tonic activity in absence of movement. The tonic putamen neurons may be the cholinergic interneurons that are intrinsic to the putamen, and thus are of interest from the standpoint of their relations to behavior.

Project Description:

Monkeys (macaca mulatta) were trained to make repeated self-paced movements for a juice reward. The delivery of the reward was preceded by the click of a solenoid valve which came to be a trigger for movements to consume the juice. Extracellular microelectrode recordings in putamen yielded a number of tonic neurons that were unrelated to body movements and that had action potentials of greater duration (mean \pm SD: 1.2 ± 0.2 ms) than action potentials of the more numerous putamen cells (0.9 ± 0.1 ms) that were silent except during movement. These tonic neurons did not at first appear to be related to any aspect of the behavioral situation. However, in raster displays of cell discharge aligned on the occurrence of the solenoid click that signaled reward it was apparent that there was a greatly increased probability of impulse occurrence approximately 60 msec following the click. This observation led to an examination of tonic neuron responsiveness in three behavioral conditions: (I) Self-Paced Movement as already described, in which a series of elbow movements resulted in a solenoid click and a juice reward; (II) Free-Reward, in which click and juice occurred at regular intervals (every 6 sec) with arm position fixed; (III) No-Reward, which was similar to Free-Reward except that the tube conveying the juice was occluded so that the solenoid click was no longer followed by juice. The paradigm involved a sequence of 40 consecutive rewards during Self-Paced Movement followed by 40 consecutive Free Rewards. Then, without altering the tempo of 6-second intervals between solenoid clicks as these clicks had occurred during Free-Reward, the No-Reward sequence of 40 clicks without juice was started. The monkeys reacted to the first few unrewarded clicks following the long sequence of 80 rewarded clicks with the motor responses (head torque and sublingual EMG) that would have been appropriate for consuming the juice, but these motor responses quickly disappeared as the series of 40 consecutive unrewarded solenoid clicks proceeded. It should be noted that the monkeys had had much experience with these three sequences, and had learned that the first unrewarded click signalled many more to come. During the experiment there were recordings of arm position and velocity, licking movements (detected by strain gauges attached to the shaft of the spout that was licked) and EMGs from sublingual muscles. Conventional extracellular microelectrode recording and behavioral conditioning techniques were used.

Major Findings:

There was no apparent difference between solenoid-evoked activity in Self-Paced Movement versus Free-Reward, showing that presence or absence of arm movements prior to the solenoid click made little difference in the tonic neuron response. Lack of reward, however, led to disappearance of tonic cell responses.

One hundred seventy-four putamen cells with tonic discharge have been studied, and 63% of these cells exhibited characteristic responses to the solenoid click preceding the juice reward, though they lacked any apparent relation to arm or licking movements. Typically, the solenoid click that was a cue for reward was followed by one impulse with a latency of about 60 msec and this single impulse was followed by a slightly lengthened interspike interval prior to resumption of spontaneous activity. Though related to the set of the monkey to consume reward, the responses in the tonic neurons were not related to licking movements per se. Other putamen neurons had bursts of discharge with each of the series

of self-paced arm movements or licking movements, but these cells lacked tonic discharge. In contrast, the tonic neurons responded to the solenoid clicks with single impulses well in advance of the first in the sequence of licking movements, and showed no apparent relation to the subsequent successive licks.

Tonically discharging putamen cells with set-dependent responses were observed at a number of loci within the putamen, but did not appear to be clustered in any particular somatotopic locus (e.g., orofacial, arm or leg as consume reward), head torque (detected revealed by movement-related putamen cells that were silent at rest and phasically active with movement of a particular body part.).

Neuronal activity in the globus pallidus was recorded in the same three click-reward contingencies, and set-dependent click-responses were observed in a number of globus pallidus neurons, but these pause disappeared soon after the No-Reward sequence started. Sixty globus pallidus cells showed set-dependent click responses and 39 were decreases of discharge.

Proposed Course:

In view of the possibility that the tonic putamen neurons may be the cholinergic interneurons of the striatum it will be important to expand greatly our information as to their neurochemistry, histological structure, anatomical distribution and behavioral correlates and this will be our goal in the coming year.

Significance to Biomedical Research and to the Program of the Institute:

Putamen cells fall into several major histological categories. The predominant cell (Type I Spiny) may account for about 90% of the neurons, but estimates of percentages are uncertain because they are dependent on Golgi preparations in which only a small proportion of cells is visualized. Data are not available as to the discharge properties of identified Type I Spiny cells during behavior, but because they are so numerous and because they are the projection neurons that send axons to the globus pallidus and substantia nigra it is commonly assumed that putamen cells that are silent during motor quiescence and that are phasically related to movement are the Type I Spiny cells. Given the different spontaneous activity patterns, the different relations to behavior and the longer duration action potentials of the tonic putamen neurons, one may hypothesize that they are not Type I Spiny Cells. Aspiny II cells are the largest neurons in the putamen, and for this reason have a fairly good chance of being sampled in extracellular microelectrode recordings in spite of making up only about 1% of putamen cells. Another category, the Spiny II cells, may be almost as large as the Aspiny II cells, but unpublished quantitative studies by DiFiglia suggest that the number of large Spiny II cells may be only one tenth the number of large Aspiny II cells. On the basis of their size and number, the Aspiny II cells would seem to be a possible source of the tonic putamen discharges that have been recorded, but it should be emphasized there is as yet no direct evidence on this point.

The classifications considered in the previous paragraph were based on the Golgi technique. Putamen cells may also be classified on the basis of neurotransmitters. The Spiny I cells are thought to be GABAergic and the Spiny II cells con-

tain Substance P. The largest cells in putamen (Aspiny II) stain for acetylcholinesterase (AChE), and recent studies using an antibody to choline acetyltransferase (ChAT) have shown that ChAT-positive neurons are large, with few dendrites and few spines. The frequency of ChAT-staining neurons in the striatum matches the frequency of AChE-intense neurons, and this correspondence supports the view that the large Aspiny II neurons, accounting for approximately 1% of all striatal neurons, are the cholinergic interneurons of the striatum. Considering the small number of these cells, one might be inclined to minimize the functional significance of the responses evoked in the tonic putamen cells. There is, however, a very important class of basal ganglia neurons containing small numbers of tonically active cells that lack phasic relations to movement: the dopaminergic neurons in the substantia nigra pars compacta of moving monkeys are tonically active and lack phasic relations to movement. By analogy, the tonic putamen cells whose activity is described in this report might have great significance in spite of their small numbers and infrequent impulses. It is clear that a high priority in further work on putamen will be the definitive neurochemical and anatomical identification of the tonic putamen neurons with set-dependent event-related responses.

Publications:

- Evarts, E.V.: Motor psychophysics: Correlations between brain cell activity and motor performance. In Borer, K.T., Edington, D.W. and White, T.P. (Eds): Frontiers of Exercise Biology. Illinois, Human Kinetics Publishers, Inc., 1984, pp. 238-243.
- Evarts, E.V.: Neurophysiological approaches to brain mechanisms for preparatory set. In Kornblum, S. and Requin, J. (Eds): Preparatory States and Processes. New Jersey, Lawrence Erlbaum Associates, 1984, pp 137-153.
- Evarts, E.V. and Wise, S.P.: Basal ganglia outputs and motor control. In Functions of the basal ganglia. Ciba Foundation Symposium 107, London, Pitman, 1984, pp 83-102.
- Evarts, E.V., Shinoda, Y., Wise, S.P.: Neurophysiological approaches to higher brain functions. New York, John Wiley & Sons, 1984, 198 pp.
- Fromm, C., Wise, S.P. and Evarts, E.V.: Sensory response properties of pyramidal tract neurons in the precentral motor cortex and postcentral gyrus of the rhesus monkey. Exp. Brain Res. 54: 177-185, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01090-08 LNP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of Central Nervous System Functional Anatomy

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Miles Herkenham	Research Psychologist	LNP, NIMH
Others:	Sandra Moon Edley	Staff Fellow	LNP, NIMH
	Ronald P. Hammer	Staff Fellow	LNP, NIMH
	Charles Gerfen	Staff Fellow	LNP, NIMH
	Rufus Gunn	Biologist	LNP, NIMH
	Candace B. Pert	Biochemist	NSB, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Neurophysiology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

5.25

PROFESSIONAL:

4.25

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects
 (a1) Minors
 (a2) Interviews
- (b) Human tissues
- (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A sensitive method for light microscopic localization of brain receptors by in vitro autoradiography was developed previously in this laboratory. By this method we have mapped the locations of opiate receptors in the brains of rats and other vertebrates, including primates. Comparisons of tritiated opiate alkaloid binding with tritiated enkephalin binding have confirmed the existence of opiate receptor subtypes. These have been related to the developing and adult dopamine system in the striatum. The possibility of pharmacological and hormonal manipulation of receptor distributions in the striatum and hypothalamus is being examined. Applications are being pursued for the concurrent study of the distributions of receptors, neuronal pathways and biologically active peptides and enzymes. The autoradiographic technique of metabolic mapping by 2-deoxyglucose has been used in functional studies of the extra-pyramidal motor system and the neuropharmacologic manipulation of this system.

I. Studies of CNS Functional Neuroanatomy. Neurochemical Investigations.

Objectives:

Over the last decade a major thrust of neuroscience research is the identification of neurotransmitter, neuromodulator and hormone receptors in the brain. An understanding of receptor function requires knowledge of the biochemistry and pharmacology as well as the neuroanatomical localization of receptors. Meaningful receptors are identified by pharmacological criteria in collaborative studies with Dr. C. B. Pert and members of the Clinical Neuroscience Branch of the NIMH. We next seek to identify the neuronal circuitry that is "plugged into" these receptors by comparison with known anatomical pathways and by immunohistochemical identification of transmitter-specific connections. Other main objectives are to understand the role of a receptor or receptor subtype in any given region by determining receptor density, maleability in tests of developmental time course or pharmacological manipulation, and altered distribution in neuropathological tissues.

Methods Employed:

We have successfully developed an in vitro autoradiographic technique for visualizing drug and neurotransmitter receptors in slide-mounted tissue slices. Fresh frozen cryostat-cut brain sections are securely attached to glass slides by a process of thaw-mounting and subsequent drying at cold temperatures. Slides are then incubated in solutions containing radiolabeled ligands. Excess and non-specifically bound ligand is washed off in cold buffered rinses, and the slides are blown dry. The sections are fixed in hot paraformaldehyde vapors under a vacuum, defatted in xylene and alcohol rinses, dried and then dipped in radioactive-sensitive emulsion for autoradiography. Alternatively, sections can be placed in an x-ray cassette and overlain with LKB tritium-sensitive film. The developed film autoradiograph then can be analyzed by a densitometer for computer-assisted quantification of receptor densities. While emulsion-coated sections provide high resolution analysis through the microscope, films can be computer-analyzed for rapid quantification of receptor densities or for color-coded image enhancement.

Major Findings:

The method we published for in vitro autoradiographic localization also appears in several books on receptor methodologies. The resolution and signal-to-noise ratio we obtain are better than other workers achieve, so we have been particularly successful at detecting subtle differences in the distributions of receptor subtypes and precise correlations with other anatomical, chemical and functional markers, including those for cells and fibers, acetylcholinesterase, labeled pathways, catecholamine fluorescence and 2-deoxyglucose metabolism. The technique is also well-suited to study fragile tissues and tissues that require suboptimal binding conditions, and so we have succeeded in studying fetal development and the binding of several "novel" peptides. These findings are outlined in detail below.

The distribution of opiate receptor subtypes, dopamine and gamma amino butyrate (GABA) receptors, and dopamine fluorescence have been compared in the mature

brain. Dopamine receptor blockers and opiate antagonists have been administered chronically to explore changes in receptor distributions and glucose metabolism in the basal ganglia. Concurrent visualization of autoradiographically-labeled projections and receptor distributions have led to understanding of functional nature of neurochemical and connectional compartmentalization within the mammalian striatum.

The ontogenetic development of striatal heterogeneity has been examined also. In the rat striatum, the distribution of mu-type opiate receptors and dopamine fluorescence appear one week before birth and take on aligned patchy distributions in a near-synchronous fashion. Whereas mu opiate receptors maintain the patchy distribution, dopamine acquires a uniform pattern during the third post-natal week. The results of combined receptor autoradiography and tract tracing in the adult suggests that the homogeneous distribution of striatal dopamine may result from the interdigitation of projections converging from several mid-brain cell groups. These projections bear a clear topographic relationship to either the mu opiate receptor-rich patches or the surrounding receptor-poor matrix, depending on their origin.

To investigate whether dopamine receptor function is important for the normal formation and maintenance of opiate and dopamine receptor distributions, dopamine receptors have been chronically blocked by the antipsychotic, haloperidol, during the prenatal stage when striatal opiate and dopamine heterogeneity develops. The *in vitro* receptor labeling technique was used quantitatively to explore the dopamine/opiate interaction in the striatum. By the day of birth, the treatment had resulted in a large decrease in striatal opiate receptor binding, increased dopamine receptor binding, and no significant change in GABA receptors. After drug withdrawal, receptor changes approach normal binding levels at different rates. Thus, the complex developmental interactions that normally occur can be disrupted by maternal administration of neuroleptic drugs.

Studies on mature striatal receptor systems are in progress to investigate several novel dopamine antagonists that produce varying degrees of antipsychotic effects and movement dysfunction.

Opiate receptors must be regarded as a dynamic rather than a static system, as illustrated by their ontogenetic appearance and sex-hormone-dependent presence in the hypothalamus. A dense concentration of mu opiate receptor binding is observed in the medial preoptic area of the hypothalamus in adult female rats during estrus and early diestrus, but not at other estrus stages, or in adult males. Moreover, dense opiate receptors first appear two days after birth in the female rat, but are never present in males unless they are castrated at birth. Thus, the opiate receptor content of this hypothalamic region, which is known to be sexually dimorphic and to affect reproductive behavior of both sexes, is hormone-dependent. Perhaps endogenous opioid peptides acting on these receptors during reproductive behavior exert a "fine-tuning" control on medial pre-optic neurons.

These findings, taken together, suggest a role for opiates in brain function that is much more complex than previously thought and indicate that further analysis of the dynamic aspects of the receptor, after pharmacological or behavioral manipulations, might enhance our understanding of its function. Ultimately,

we hope to determine the role that opiates and related neurochemicals play in human brain function, and separate out the striatal chemoarchitecture that is involved in some psychiatric and neurologic syndromes.

II. Metabolic Correlates of Functional Activity

Objectives:

We have developed high-resolution autoradiographic techniques for the localization of metabolic activity at the light microscopic level. Patterns of metabolic activity marked by [³H]2-deoxyglucose uptake have been compared in normal, alert rats to those of animals given various drugs systemically or intracerebrally at specific loci. Using series of adjacent tissue sections from a single animal, patterns of metabolic activity during drug administration can be correlated with the localization of receptors to which the drug binds.

Methods Employed:

We have developed techniques which permit us to use [³H]2-deoxy-D-glucose (2-DG) as a metabolic marker of glucose utilization, visible at the cellular level of resolution. Low resolution was a persistent problem in previous autoradiographic localization studies which utilized [¹⁴C]2-DG as a marker. The use of [³H]2-DG improves resolution, since the particles of ³H are less energetic than those of ¹⁴C and form an image closer to their source.

Autoradiographic localization of brain receptors can be compared in the same animal with manipulation-induced alterations in brain metabolism measured by the 2-DG technique. Alternate sections from an animal previously injected with 2-DG are either processed for 2-DG autoradiography as described or for receptor localization. The latter is accomplished by first removing the diffusible 2-DG in preincubation solutions prior to in vitro receptor binding. In this way, the alteration of brain metabolism by drugs or anesthetics may be correlated with receptor binding in those brain regions affected.

Major Findings:

Our studies of phencyclidine-induced changes in cortical metabolism elucidate the mechanism of action of phencyclidine analogs in the central nervous system. Metabolism in regions of limbic cortex is stimulated by these drugs while sensory cortical zones show decreased metabolic activity. This inhibition of glucose uptake occurs in all layers except layer Va of primary somatosensory and primary visual cortex; however, activity in secondary cortical regions is spared. Correlative 2-DG and receptor binding studies have shown that the cortical regions which show decreased metabolism also contain higher densities of GABA receptors. A phencyclidine-induced GABAergic influence on these specific cortical zones may be a factor in the decline of cortical sensory metabolism.

We have begun to investigate the functional role of monoamine neurotransmitters in the extrapyramidal motor system, using 2-DG techniques. Depletion of monoamine input to the striatum by chronic reserpine administration causes a metabolic activation of the globus pallidus, a structure which receives afferent projections from the striatum. Reserpine depletes dopamine in the striatum and

alters neuronal activity in both the striatum and pallidum. The presumptive disinhibition of pallidal activity leads to increased metabolism at this locus. Reserpine also induces elevated 2DG labeling in the lateral habenula, another structure in the extra-pyramidal circuitry. Systemic injection of L-DOPA, a dopamine precursor, results in a rapid reversal of these metabolic alterations. These results suggest that dopamine directly influences striatal outflow. Current work explores the mechanism of these metabolic changes in the extrapyramidal motor system. The pattern of metabolic labeling following the injection of inhibitory neurochemicals into specific extrapyramidal loci should tell us whether altered 2DG labeling in distant loci results from postsynaptic neuronal activity or from activity in the afferent terminals.

Significance to Biomedical Research and to the Program of the Institute, and Proposed Course:

The visualization by autoradiographic techniques of opiate receptor locations throughout the CNS has greatly advanced our appreciation of the richness of opiate functions in normal physiology and has opened a surprising number of doors to the investigation of receptor-mediated brain processes. We have just begun to appreciate how receptors influence and control neuronal development and the establishment of neural connections, the interrelatedness of receptor subtypes and of neurochemically distinct systems (such as dopaminergic and opiate interactions in the striatum), the evolution of receptors as markers of synaptic complexity and the significance of species differences. We are encouraged by comparisons of drug receptors and the altered metabolic profile (as seen by 2-deoxyglucose autoradiography) produced by the same drugs. These findings indicate a productive future in the research of brain function. Applications of these techniques for the study of alterations in the brains of deceased humans with histories of mental disorders are being evaluated.

Publications:

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- Goldstein, B., Maxwell, D.S., Ellison, G., and Hammer, R.P.: Dendritic vacuolization in the CNS of rats after long-term voluntary consumption of ethanol. J. Neuropathol Exp. Neurol. 42: 579-589, 1983.
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van der Kooy, D., Koda, L.Y., McGinty, J.F., Gerfen C.R., and Bloom, F.E.: The organization of projections from the cortex, amygdala and hypothalamus to the rat solitary complex. J. Comp. Neurol. 224: 1-24, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
 NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

201 MH 01091-07 LNP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Motor Function in Patients with Neuropsychiatric Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Jerome N. Sanes	Staff Fellow	LNP, NIMH
Others:	Edward V. Everts	Chief	LNP, NIMH
	Von A. Jennings	Staff Fellow	LNP, NIMH
	Karl-Heinz Mauritz	Guest Worker	LNP, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Neurophysiology

SECTION

INSTITUTE AND LOCATION

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TOTAL MAN-YEARS

2.0

PROFESSIONAL:

2.0

OTHER

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- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purposes of this project are to examine the contributions of central motor programming and afferent input in control of arm movements in normal subjects and patients with sensori-motor disorders, and to study psychomotor performance of patients with central motor disorders. The first set of experiments records muscle activity and kinematics of limb position while (1) subjects manually match a target display with either a skilled rapid or slow movement with a handle whose displacement controls a visual display or (2) maintain postures when limb position is passively changed. Movement amplitude, presence or absence of visual feedback of position, disturbances of the subject's movements and changes in sensory input are independent variables. Large movements are performed accurately independent of manipulation of the experimental variables but accurate performance of small movements becomes increasingly dependent on the absence of limb disturbances during movement. The second set of studies examined a variety of psychomotor variables from patients with a variety of neurological disorders. Voluntary and involuntary movements are evaluated to develop sensitive measures of psychomotor performance that correlated with clinically determined fluctuations in drug efficacy.

I. Central and Peripheral Control of Movement in Humans.

Project Description:

The importance of afferent information in the control of limb movements is controversial. Whereas it is clear that afferents exert potent physiological effects on spinal motoneurons and cells in supraspinal structures, it has been suggested that some of these afferents contribute little to the final positioning of a limb. A case in point is the observation that muscle spindle activity does not reflect muscle length during rapid movements of large amplitude, thereby casting doubt on a regulatory role for spindles at the end of movements. In addition, physical disturbances imposed during movements, that likely activate muscle spindles, do not appear to modify final limb positioning. There are, however, other experiments demonstrating the importance of afferent input in a variety of tasks performed by humans. For example, ischemic deafferentation of limbs alters position sense and sense of effort. Furthermore, performance of fine motor tasks, such as reproduction of alphabetic characters is also disrupted by ischemic deafferentation and it is noteworthy that inactivation of the gamma loop in humans impaired the ability to tonically activate motor units, though phasic activation was not impaired. It is the object of the present project to continue examination of the role of peripheral inputs in the control of limb movements and postural control. In addition, the organization of central processes of movement will be explored further. In many of these experiments the trajectories and end-points of two-dimensional movements about the elbow and shoulder will be evaluated. Both normal volunteers and patients with neurological disorders will be studied during performance of movements of varying sizes and when a maintained posture is disturbed by different peripheral inputs. Different types of limb disturbances will be imposed during the movements. Two general experimental approaches are being pursued. In the first, the psychomotor variables of movement error and movement time are studied in relation to physical disturbances. In the second group of experiments, electromyographic activity is examined when the limb is mechanically perturbed while subjects perform motor tasks or maintain postures.

Methods:

Human subjects are trained to manipulate a handle that is attached to a servo-controlled torque motor while performing extension-flexion of the wrist, elbow or index finger, or abduction-adduction of the index finger. Displacement of the handle causes movement of an oscilloscope beam that is to be matched by the subject with a second, experimenter controlled, oscilloscope beam. In one series of experiments, subjects perform tracking movements either as rapidly as possible or as accurately as possible. For a variety of movement sizes (3° to 30°) subjects are given an adequate number of training trials. Independent variables include (1) continuous loads opposing or assisting movement, (2) brief physical disturbances delivered to the arm before or after initiation of arm movement and (3) initial starting position and (4) full or partial information concerning the direction and extent of the instructed movement. Patterns of muscle activity and tracking errors are analyzed during rapid movements.

In the two-joint experiments, subjects will be asked to grasp a handle that is attached to a mechanical arm that also rotates at what would be elbow and shoulder joints. With this apparatus it is only possible to perform movements in the horizontal plane. The shoulder joint of the mechanical arm can be prevented from moving with an electrically activated clutch.

Preliminary Findings and Concepts:

Motor behaviors during one and two-dimensional movements of normal subjects and patients with large fiber sensory neuropathy have been studied. Several findings have emerged:

(1) When normal subjects perform planar two-dimensional movements with the elbow and shoulder the hand moves through space in a straight line without discontinuities. Thus, the motor control system provides an optimal signal for movement between two points in space. In addition, the onset time, peak velocities and termination of elbow and shoulder displacement are nearly identical; thereby indicating a close coupling of shoulder and elbow during the dynamic phase of movement. The hand trajectories of patients with a large-fiber sensory neuropathy are smooth and without discontinuities but the trajectories have more curvature than those of normal subjects. Seemingly, kinesthetic inputs are important in the development of optimal signals for voluntary movement. From these results it was not clear whether humans need to monitor kinesthetic information only at the beginning of movement or require continuous updating of limb position during movement.

(2) The hierarchical organization of limb motor control can be explored by evaluating two-dimensional movements. In particular, it is of interest to determine whether movement control is organized on an "object" level or a "joint" level. Object level organization implies that brain signals control the hand position by a coordinated and interdependent control of the elbow and shoulder joints. Alternatively, joint level control would entail relatively independent control of the elbow and shoulder joints. As noted earlier, the elbow and the shoulder movements seem to be coupled during the dynamic phases of movement. However, it is unclear whether this dynamic coupling extends to end-point control. A means of distinguishing between object level or joint level motor control is to evaluate the shape of the error region, that is the variability in hand position in Cartesian coordinates, at the end of movement in which the elbow and shoulder are used in varying proportions. The expected error for joint level control would be related to the proportionate amount of angular displacement of shoulder and elbow. That is, equal angular displacements of shoulder and elbow would result in a circular error region reflecting random errors in both the shoulder and elbow displacements. However, the error region for unequal angular displacement of shoulder and elbow would be elliptical with the long axis of the ellipse reflecting error of the joint with the larger displacement. The error region for object level control would always be circular, thereby reflecting the linked control of the elbow and shoulder. The data from preliminary experiments with normal subjects indicate that a joint level signal is sent to the elbow and shoulder muscles since the error region appears to vary as a function of the relative displacement of the shoulder and elbow.

(3) A third concept to be investigated in the central organization of multi-joint movements is the extent to which muscle activities of individual joints participating in the movement are coordinated. It is quite clear that muscle activity during rhythmic movements, such as locomotion and reflexive scratching, is highly stereotyped and reflects the operation of central pattern generators. The two-dimensional movements evaluated in the current project are not stereotyped but instead are infinitely variable. Since the dynamic phases of elbow and shoulder movement are coupled it might be expected that muscle activities in the two limbs would exhibit comparable patterns. On the other hand distinctive electromyograms in shoulder and elbow muscles may provide insights into the variability in end-point position of the hand.

(4) In separate investigations, the reaction time (RT) performance of parkinsonian patients was evaluated. Four procedures were used to investigate whether the amount of information available about the impending movement before a "go" signal was processed normally by patients with Parkinson's Disease (PD). In the first test, patients held an electrical stylus that was moved in 20 individual movements between sets of targets that varied in width from 0.5-4 cm. The distance between the targets was 4-32 cm. Movements by normal subjects were required to be within a strict accuracy criterion, whereas patients were encouraged to move as accurately and as rapidly as possible. Variation of movement amplitude and target width altered RT and MT of both normals and patients with PD. For normals and patients RT increased as the required movement became more difficult; that is as movement size increased or target size decreased. The increase in RT was steeper for PD patients. The performance of PD patients was slower and less accurate than normals but there was also a steeper rate of change in MT as movement size increased for the various target widths. The greater slope of MT/cm for PD patients for all target widths was related to substantial increases in MTs for the largest movements. These findings demonstrate that changes in movement size and target width will modify RT and MT in PD patients differently from that of normal subjects. The primary deficit for PD patients was the failure to initiate movement quickly and to accurately execute large amplitude movements. Thus, as the index of difficulty increases (independent of the source of the increase) patients with PD react and move slower than normals. Therefore, equations relating RT or MT, accuracy and movement amplitude differ between normals and patients with PD. Since emphasis was given to rapidity, as well as accuracy, of movement it may be expected that patients with PD would perform reasonably well in tasks of this type if they are given ample time to complete a movement.

For a second series of tests, RT was measured when patients performed in a choice reaction time task, and a two-movement delay task. For the choice RT task there were four possible movement locations and patients viewed either 0, 1, 2 or 4 "pre-targets" that indicated the direction and extent of the next movement. If only 1 pre-target was present the patient knew both the direction and extent of the next movement. However, when 2 or 4 pre-targets were presented, there was uncertainty about the next movement until the "go" signal was presented. In this situation, parkinsonian patients showed increased RT when the behavioral choice was made upon appearance of a "go" command. In another test, patients were required to make two movements, with the second movement occurring with a 0-1000 msec delay after initiation of the first movement. In normal subjects,

the RT of the second movement is similar to the RT for the first movement. The period between the two RTs has been referred to as the psychological refractory period. The observation of similar RTs for the first and second movement implies that the mechanisms responsible for generating movement require a minimum processing time but are not delayed significantly by prior occurring processes. Thus, it was of interest to determine in PD patients whether performance of one movement would interfere with initiation of a second movement. It was found that for PD patients the second RT was greater than the first RT, indicating that PD patients are impaired in executing sequential motor responses.

Proprioceptive Inputs and Motor Control

In previous annual reports, we described preliminary findings on the role of proprioceptive information in movement control of normal humans and patients with sensory disturbances. The results demonstrated the importance of information derived from large fiber somesthetic afferents for postural stability, force maintenance, performance of discrete movements, especially those of small magnitude, and in the development of enhanced physiological tremor. The importance of somesthetic inputs was particularly apparent in the ability to maintain constant motor output as shown by the failure of "deafferented" patients to sustain a given level of muscle activity or maintain postural stability. The postural instability of deafferented patients was evident, though diminished, even when visual guidance was available. But upon removal of visual guidance of hand position, patients had no sensory feedback for guidance and any postural drift remained uncorrected. The deterioration of postural maintenance of deafferented patients was typically observed soon after (< 2 seconds) the visual guidance had been removed. It was as if the brain structures controlling levels of excitation to the muscles "forgot" the appropriate level of motor neuronal excitation upon elimination of visual guidance, with the result that motor commands began drifting when visual guidance was eliminated. This rapid deterioration of steady-state output levels suggests that motor memory requires updating, presumably by kinesthetic afferents, of the consequences of intended motor output. In addition to deficits in posture, phasic motor control was also impaired in the patients with sensory loss since discrete movements were poorly performed. Without vision, the patients would typically overshoot or undershoot the target and then briefly maintain the new position. Thus, it appears that the termination of discrete movements also depends on somesthetic afferent input.

The studies with normal subjects that were concerned with the role of proprioceptive information in movement control indicated that unexpected mechanical perturbations occurring at the beginning of movement disrupted accuracy of small movements more so than the accuracy of large movements. These behavioral results occurred despite the observation that larger "compensatory" muscle responses were triggered by the perturbations occurring during large movements. It might have been expected that the absolute size of the triggered muscle response would be related to accuracy but instead the relationship between the voluntary and triggered muscle responses are critical in determining whether an accurate end-point position will be achieved. Since small movements are caused by small amounts of muscle activity compared to the electromyogram seen with a maximum contraction it could be stated that a relatively small proportion of the motor neuron pool is activated by a motor command signaling a small movement. In

contrast, large movements are caused by relatively large amounts of muscle activity compared to that observed with a maximal voluntary contraction and a relatively large portion of the motor neuron pool is recruited by a motor command signaling a large movement. Thus, small movements probably recruit only low threshold motor units while large movements recruit low and medium threshold motor units. An additional observation on the organization of the motor neuron pool suggests why small movements are more affected by perturbations. That is, the motor units in any pool with relatively low thresholds are obviously more easily recruited and probably easier to modulate in discharge rate than high threshold motor units. If only low threshold motor units are recruited for small movements excitatory peripheral inputs would be more likely to recruit motor units with slightly higher thresholds. In contrast, peripheral inputs occurring during large movements would have to raise the excitability level of the motor neuron pool more to recruit new motor units than if small movements were performed. Therefore, the summation of the motor command and the triggered EMG reaction would appear to be the important variable in determining whether movements were accurate or inaccurate.

Significance to Biomedical Research and to the Program of the Institute:

Additional clarification of how somatosensory information and central motor commands are used to control skilled motor activity is essential to the understanding of normal and abnormal motor behavior in humans.

Furthermore, these studies will provide standards of normal motor function and allow comparisons with patients with motor disorders to evaluate subclinical deficits and the efficacy of pharmacotherapeutic agents. The objective evaluation of neuropsychiatric disorders that we have developed should prove useful in a wide variety of experimental applications that require computer recording and analyses of results. Long-term evaluation of patients' progress on medication regimens is particularly suited for objective analysis.

Proposed Course:

Future studies concerned with psychomotor performance will continue to investigate the importance of tactile and kinesthetic signals occurring during movements. Thus, properties of movements and muscle activity will be investigated in normal and fatigued muscles, from functionally deafferented limbs, and following vibratory or mechanical disturbances delivered to a limb. A variety of movement types (e.g. large/small) and strategies (e.g. fast/slow) will be studied to determine the movements that depend upon sensory inputs from the periphery for accurate completion. More than two years of experimentation will be required to validate and extend the preliminary findings in providing additional information on the control of limb movements in normal and neurologically diseased humans.

Publications:

Sanes, J.N., and Evarts, E.V.: The regulatory role of proprioceptive input in motor control of phasic or maintained voluntary contractions in man. In Desmedt, J.E. (Ed.): Motor Control Mechanisms in Health and Disease. New York, Raven Press, 1983, pp. 47-59.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 01092-06 LNP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Non-Primary Motor Cortex and the Cerebral Control of Movement

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Steven P. Wise Research Biologist LNP, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

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SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS

0.8

PROFESSIONAL

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- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project grew out of our neurophysiological and neuroanatomical investigation of the corticocortical connections in the somatic sensorimotor cortex of monkeys and their role in the control of primate motor behavior. In that project we re-defined three of the main cortical inputs to the primary motor cortex (MI): the premotor cortex (PM), the supplementary motor cortex (MII) and the transition zone between the motor and somatic sensory cortex, area 3a. These three cortical fields surround MI and can be differentiated from MI on the basis of neuronal responses to peripheral inputs, thresholds for evoking movements with intracortical electrical stimulation, the properties of single neurons during the performance of an operantly conditioned motor task, cytoarchitecture, and connectivity. We then elaborated this study by an analysis of the activity of single neurons in PM during a variety of visually guided motor tasks. Each of these behavioral tasks was designed to elucidate the role of PM in the cerebral control of movement. We have tested the following hypotheses: that PM guides movement to points in space, that PM is involved in the sensory guidance of movement, that PM plays a role in motor preparation that it functions in the determination of movement parameters, that it reflects eye position, gaze position, postural muscle activity, visual fixation, attention, motivation, or arousal. Of these ideas, our results support the hypothesis that PM plays a role in the execution of visually guided movements and the preparation for voluntary movements.

Objectives:

The inputs to the precentral motor cortex (MI) and its intrinsic neuronal circuitry determine the output of MI neurons, including those projecting to the spinal cord. We hope to gain an understanding of the inputs to MI cortex and their interaction in producing motor cortex output. The long-term objective of this project is to examine the activity of neurons that project into and out of MI and to contrast the functional significance of corticocortical and corticofugal neurons. Our colleague on this project, this year, was Dr. Karl-Heinz Mauritz, a Visiting Scientist and before the fiscal year is over we will be joined by Dr. Kiyoshi Kurata, a Visiting Fellow of the Fogarty Foundation.

Two more general objectives of this project are (1) an improved understanding of the organization of the entire motor cortex, a region which is likely to include, in addition to its "core," the MI cortex, a surrounding neocortical "belt" containing two or more representations of the motor periphery and (2) a better understanding of the cortical fields involved in the sensory guidance of movements and the linkage between sensory signals and motor behavior.

Methods:

Seven monkeys have been trained to perform several visually guided motor tasks. Each task is considered to be a separate experiment. (1) A rhesus monkey was operantly conditioned to depress one of four keys located in a perimeter at arms length. While the monkey pressed one key, another of the four keys, selected randomly was illuminated after a randomly varied delay. This key thereby became the next target. A barely discernable visual cue near the target key, appearing after another variable delay, signaled the monkey to move and depress the target. The monkey was required to make the movement within a short period of time, near the limit of reaction time. Neurons in the premotor cortex were studied in this experiment. (2) The monkeys were conditioned to align two spots of light on a tangent screen in front of the monkey. One of these spots is controlled by the computer (the target light), the other by arm movements of the animal (the position light). The monkey was required to align the spots within a small accuracy "window." In five-sixth of the trials, after a short period of time the target light jumped to one of six locations. The monkey had to maintain his arm position unchanged until the target light dimmed, at which point he was required to flex or extend his forearm rapidly and accurately. In one-sixth of the trials, the computer selected a situation in which physically identical stimuli signaled the animal to make no movement. This experiment was designed for two purposes: to contrast neuronal activity in MI and premotor cortex and to distinguish neuronal activity when identical stimuli signal the execution or withholding of movement. (3) The monkey was conditioned to depress the central key of three keys. After a period of time, either the left or right key became illuminated. Three experimental conditions ensued: (a) the key remained illuminated and served as the target for the subsequently triggered movement, (b) the light was turned off before the monkey was allowed to execute the movement, forcing the monkey to remember the proper target, or (c) the target light was switched before the monkey was allowed to execute the movement. This experiment was designed to further test the relationship of neurons in PM to the motor set of the animal, even when the signals are absent or the motor set changes during the course of a trial. (4) Monkeys are being conditioned to execute simple movements as

well as short sequences of movement. The monkey is seated in front of a panel of three keys as in experiment #3. Each trial starts with the monkey pressing the leftmost of the three keys. Two experimental conditions then ensue: (a) the center key is illuminated, thus indicating that a simple movement is to be made to depress the center key, or (b) both the center and right lights are simultaneously illuminated to indicate that a short motor sequence should be initiated to depress both keys, in order.

The single unit activity and behavioral data were collected on-line with a PDP 11/03 computer and analyzed off-line with a PDP 11/34 computer. Many of the routines used in off-line data analysis were developed by W. Sheriff of the Research Services Branch.

Following the recording procedures, small amounts of [³H]-amino acid may be injected into either the premotor cortex, MI or MII. By noting the ultimate distribution of radioactivity in the brain, the sites of termination of neurons in the somatic sensorimotor cortex can be determined by tissue autoradiography.

Experimental Findings:

About 1400 units have been examined in this project to date, and 666 of these have been studied in detail. Several findings and interpretations have been developed:

1. MII and premotor cortex neurons are virtually unresponsive to peripheral somatosensory inputs, compared with MI neurons in the same monkeys. This finding is somewhat surprising from a neuroanatomical perspective, since MII receives monosynaptic corticocortical input from most subdivisions of the somatic sensorimotor cortex and premotor cortex has a variety of potential somatosensory inputs from cortical regions. However, the lack of profound somatic sensory responsiveness supports the hypothesis that MII and premotor cortex play a role in centrally generated motor programs rather than movements regulated by peripheral feedback.
2. These and additional findings have enabled us to improve the current understanding of cerebral localization in this part of the cortex, notably the relationship of physiologically defined cortical regions to those defined by anatomical methods. Two of these points are most noteworthy: (1) Microelectrode methods reveal that the boundary between MI and MII corresponds to the boundary between two anatomically defined parts of the agranular neocortex (termed areas 4 and 6 by Brodmann in 1909). The boundary between MI and premotor cortex corresponds not with the boundary between areas 4 and 6 drawn by Brodmann (1909) but rather an analogous boundary of von Bonin and Bailey (1947). (2) Area 3a, the transitional field between the agranular and the highly granular somatic sensorimotor cortex, appears to be, as it was originally defined (in the work of C. Vogt and O. Vogt, 1919) a discrete cortical field characterized by a thin internal granular layer (layer IV).
3. Our study of premotor cortex has shown that most neurons in that cortical field change activity markedly before the onset of a voluntary movement. Their activity is often specific for the direction of arm movement. These neurons

are located within the frontal agranular cortex, corresponding to a part of area 6 as defined by the absence of a large population of giant, layer V pyramidal cells in addition to the lack of an internal granular layer (layer IV). The premotor cortex can also be distinguished from the MI representation by its markedly increased threshold for evoking movements with intracortical microstimulation. Further, a substantial population of neurons change their activity in relation to motor set and/or signals which indicate the location of motor targets. One class of cell in premotor cortex, termed "set-related neurons," appear to be specifically correlated with the motor preparation (or set) of the animal. This hypothesis has been supported in three ways: (a) these units show changes in activity when visual signals cue a movement, (thus establishing a specific motor set), but not when the same signals instruct the monkey to withhold movement, (b) when the guiding visual signal changes (to establish a different motor set), the unit activity changes to reflect the new set, and (c) when the guiding signal is removed (but the set remains the same), the unit activity continues to reflect the motor set rather than the sensory signals. In addition, it has been found that these and other premotor cortex units change their activity in relation to predictable environmental events.

Significance to Biomedical Research and to the Program of the Institute:

The activity of higher-order motor cortical fields such as premotor cortex is important to the understanding of the cortical control of motor acts of the least automatic kind, in both health and disease, and especially for understanding the way in which sensory signals are converted, by the brain, into organized motor acts. A much improved knowledge of the non-primary areas of the cerebral cortex is essential to an understanding of higher brain functions of all types.

Progress and Proposed Course of the Project:

The past year has been devoted to conducting experiment #3 (see Methods) and writing two full-length reports of its results, completing publication of the data obtained in experiment #2, and writing two scholarly reviews on the subject central to this project. Two other, shorter reviews were also written this year. Also of relevance to this project was the writing and completion of a monograph, based partly on the data obtained and ideas developed in the conduct of this project. In addition, animals were trained for experiment #4, and a future experiment aimed at an investigation of the topographic organization of premotor cortex was partially designed, this year. In order to further examine the functional organization of motor cortex we must acquire more knowledge about the input-output organization of the cortex and the differential roles of the various fields within the somatic sensorimotor cortex, especially those involved in higher-order control of movement. There is also a need for more reliable methods with which to distinguish the frontal cortical fields from each other, especially in awake behaving animals. This project will be continued and developed in the Laboratory of Neurophysiology with the collaboration of Dr. K. Kurata, a Fogarty fellow.

Publications:

Evarts, E.V., Shinoda, Y., and Wise, S.P.: Neurophysiological Approaches to Higher Brain Functions., John Wiley and Sons, New York, 1984, 198 pp.

Weinrich, M., Wise, S.P., and Mauritz, K.-H.: A neurophysiological analysis of the premotor cortex in the rhesus monkey. Brain 107: 385-414, 1984.

Wise, S.P.: The primate premotor cortex. Shinkei Kenkyu no Shinpo, 28: 58-66, 1984.

Wise, S.P.: Non-primary motor cortex and its role in the cerebral control of movement. In Edelman, G., Cowan, W.M. and Gall, E. (Eds.): Dynamic Aspects of Neocortical Function. New York, Neurosciences Institute, John Wiley and Sons, 1984, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 01093-06 LNP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Role of Somatic Sensory Inputs in the Cerebral Control of Movements

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Von Jennings Staff Fellow LNP, NIMH

Others: Steven P. Wise Research Biologist LNP, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

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INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS

1.1

PROFESSIONAL

1.1

OTHER

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project examines how somatic sensory inputs to the precentral motor cortex (MI) and somatic sensory cortex (SI) influence motor behavior in primates. In particular, the hypothesis that MI neurons respond to mismatch between actual and intended movements was examined by stopping wrist movements in monkeys seeking to make accurate displacements. It was found that the response of MI neurons to stop depends on the distance between the stop position and intended terminal position. It is concluded that, during a perturbation, MI activity reflects the magnitude of the deviation from the intended displacement. This finding provides an important clue concerning the role of peripheral inputs to motor cortex in the initiation and control of movement.

Objectives:

MI neurons are known to receive inputs from a variety of receptors in joints, muscles and skin. However, the significance of these inputs remains unclear. One possible role may be to drive motor cortex output in response to a mismatch between actual and intended movement. While it has been shown that the sign of the MI response to a perturbation is appropriate to produce a compensatory muscle response, the quantitative relation between magnitude of cortical responses and extent of mismatch has not been systematically studied.

Another question of considerable importance concerns the route by which peripheral information reaches MI neurons. Previous experiments in this project addressed this question by studying the activity of SI neurons in regions that are known to be densely and reciprocally connected to MI. MI neurons and neurons in posterior SI were found to be strikingly similar in their relation to actively held limb posture and exerted force. Thus, it is possible that peripheral inputs to MI are relayed through SI. Further evidence for this possibility is being sought by comparing the pattern and timing of MI and SI responses to perturbations during active movement. This information will be of significant theoretical importance concerning the role of sensory feedback to the sensorimotor cortex in the initiation and control of voluntary movement.

Methods:

The basic paradigm in this project involved conditioning monkeys to use voluntary wrist flexion and extension displacements to move a handle that was attached to a servo-controlled torque motor. During some of the movements the torque motor was used to halt the displacement for 200 ms. Two movement amplitudes (7° and 15°) were performed. In addition, these movements could be stopped either early (3° from the beginning) or late (3° from the end).

Activity from single MI and SI neurons and codes specifying certain behavioral events such as movement onset and stimulus presentation were recorded on-line with a PDP-11/03 computer. Analog signals of handle position, velocity and torque and muscle activity from wrist flexor and extensor muscles were recorded on magnetic tape. Off-line analysis of single unit activity with a PDP-11/23 computer involved a comparison of the pattern and frequency of neuronal discharge during stopped and unstopped movements. A Data Precision 6000 signal averager was used to compare the rectified and filtered muscle activity during these same movements.

Experimental Findings:

Effects of Stop on Muscle Activity

1. The monkeys' control (i.e. unstopped) movements were associated with a triphasic electromyographic (EMG) pattern and all phases of this pattern were affected by stops.
2. Increase in agonist EMG and decreases in antagonist EMG occurred at latencies of 15 ms - 45 ms from stop onset.

3. Stops near the beginning of movements caused greater magnitude stop responses than stops near the end even though movement velocity was less at the time of the early stop.

4. It is concluded that the magnitude of the EMG response to stopping voluntary movement depends critically on the distance between the stop position and the intended terminal position as well as on the phase of the movement at which the stop occurs. Both of these findings provide evidence that the quantitative aspects of the stop response of muscles reflect the magnitude of the mismatch between actual and intended position.

Effects of Stop on Single Unit Activity

1. Many MI and SI neurons displayed stop related activity that resembled the pattern of activity seen in prime mover muscles during stops. For example, some neurons related to flexion movements increased their activity when flexion movements were stopped but showed no change or decreased activity during stops of extension movements. In addition, these neurons increased their activity more during early stops of flexion movements than during late stops.

2. The onset latency of the neuronal response to stop ranged between 18 ms and 80 ms for MI neurons. SI neurons with stop responses strikingly similar to those seen in MI were found in area 3a and area 2. Few were seen in area 3b or area 1. While no large differences between the response latencies of MI and SI neurons have been observed, a more quantitative analysis is in progress to determine if a small but significant difference exists.

3. Several MI neurons were seen whose activity during stops did not resemble the stop response of any studied muscle. With few exceptions, however, the activity of these neurons also did not resemble the pattern of muscle activity during unperturbed movements. In contrast, the activity of several SI neurons was found to resemble prime mover muscle activity during unperturbed but not perturbed movements. A possible explanation for this finding may be that many types of input evoked by a stop reach SI but only those inputs appropriate to produce a compensatory muscle response are relayed to MI.

Significance to Biomedical Research and the Program of the Institute:

In order to better understand normal and abnormal limb movements in man more information is needed concerning how inputs from the periphery are used to initiate and control skilled motor activity. While it is known that the motor cortex receives peripheral inputs prior to and during limb displacements, the function of these inputs remains to be clarified. An examination of this question will contribute to our understanding of the role of sensory feedback in the cerebral control of movement:

Proposed Course of Project:

While the findings presented in this report are consistent with the hypothesis that MI activity reflects the magnitude of mismatch between actual and intended position, the most salient features of the mismatch remain to be clarified.

The next step in our experiments will be to determine which aspects of a deviation from intended movement, such as distance, velocity or acceleration, contribute most to the observed change in cortical activity.

Publications:

Sanes, J.N. and Jennings, V.A.: Centrally programmed patterns of muscle activity in voluntary motor behavior of humans. Exp. Brain Res. 54: 23-32, 1984.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 01094-04 LNP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Information Processing in the Motor Cortex

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: John P. Donoghue Staff Fellow LNP, NIMH

Others: Steven P. Wise Research Biologist LNP, NIMH

COOPERATING UNITS (if any)

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Laboratory of Neurophysiology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

1.1

PROFESSIONAL:

1.1

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is to examine the input-output organization of neurons in the motor cortex. Rats have been chosen as the primary experimental animal since they are readily available and have small, lissencephalic brains. Neuroanatomical and neurophysiological techniques are being employed to characterize the primary motor cortex (MI) and other closely related cortical areas in rats and determine their connectional relationships with other neural structures. Immunocytochemical techniques are being used to identify the transmitters of cortical neurons. Neuronal activity will be monitored in the forelimb area of MI cortex in awake, behaving rats and the contribution of inputs to these neurons in generating or modulating discharge patterns during movements will be assessed. Intrinsic cortical circuits are being studied by intracellular injection of a tracer, horseradish peroxidase, to demonstrate the connectional relationship between intrinsic and projection neurons in the cortex.

Objectives:

The output of motor cortex neurons is closely linked to motor activity in mammals. These cortical outputs arise after intracortical processing of inputs through a complex array of neurons. The experiments described here are designed to identify the input-output transformations that occur in the first motor (MI) subdivision of somatic sensorimotor cortex.

Our objectives are to identify each source of input to MI and the axonal targets of MI neurons by employing neuroanatomical pathway tracing techniques, to identify the transmitters used by some intrinsic and cortical projection neurons, to examine the activity of neurons in MI during motor behavior (a simple forelimb motor task), and to study aspects of the synaptic circuitry within MI. The overall objective is to gain a better understanding of routes of information flow within small modules of neocortex.

Methods:

1. Identification of inputs and outputs of MI cortex. For pathway tracing experiments, axonal tracers (histochemical markers or radioactive amino acids) are injected into neurophysiologically characterized cortical regions. Following appropriate survival times the animals are perfused and the brain processed by standard methods to reveal the distribution of tracer substances in the brain and thereby the connections of the injected cortical regions.
2. Identification of cortical transmitters. The transmitters of cortical neurons are identified with immunocytochemical techniques. Antibodies to synthetic enzymes of putative neurotransmitters are applied to tissue sections from rats and the distribution of labeled neurons demonstrated by standard immunocytochemical techniques.
3. Chronic single-unit recording. Rats are trained to press a bar with their forelimb in a stereotyped manner in order to obtain a water reward. After learning the motor task, a recording chamber is placed over the forelimb region of MI cortex and stimulating electrodes are placed: (a) in the locus coeruleus to stimulate these cortical afferent pathways or (b) in the pyramidal tract in order to identify cortical projection neurons. Subsequently, single unit recordings are made during task performance. In other animals an electrode that allows both single unit recording and injection of putative neurotransmitter agonists and antagonists is used to identify the role of these transmitter candidates in the control of neuronal activity in cortex. Unit activity, force, and occurrence of bar pressing are recorded online with a PDP 11/03 computer and these data are analyzed off-line with a PDP 11/34 computer.
4. Intracortical circuitry. The intracortical connections of neurons in MI cortex are identified by intracellular recording and injection of the tracer, horseradish peroxidase (HRP). Intracellular recordings are made with glass electrodes filled with 4% HRP in tris/KCl buffer. Certain distant axonal connections of each neuron may be determined with antidromic activation methods. In some cases one class of neurons (projection or intrinsic) will be labeled by intracellular injection of HRP and another class of projection neuron will be labeled with

retrograde transport methods. This double labeling scheme will allow examination of the connectional relationships between two sets of identified neurons in cortex.

Experimental Findings.

1. Cortical field definition and pathway tracing experiments. With intracortical microstimulation and axonal transport methods, we have shown that the MI cortex of the rat coincides with a distinct cytoarchitectonic area, the lateral agranular field (AG_1), and also includes part of the adjacent granular cortex of the first somatic sensory area. We have now examined the inputs to AG_1 with axonal transport methods. We have found that AG_1 receives input from cortical somatic sensory areas and subcortical regions involved in motor control. This combination of inputs suggests that AG_1 is an important cortical region for sensorimotor integration and movement control. There are further inputs from the basal forebrain region, the midbrain raphe nuclei and the locus coeruleus. These systems are likely to have a modulatory effect on cortical output.

The outputs of motor cortex are currently being examined. We have discovered that MI in the rat has dense connections to the structure most directly involved in movement control, the ventral horn of the spinal cord. In addition, motor cortex in rats projects to other motor-control structures, such as the pons and the striatum. MI also projects back to cortical and thalamic centers that provide its main input. This suggests that MI may control the ascending information it receives.

We have also examined the connectional relationship between the cortex and another important motor control structure, the neostriatum. These studies have shown that there are multiple patterns of corticostriatal projections that vary according to the cortical area from which they originate. Furthermore, these projections have specific relationships to opiate receptor rich patches in the striatum. Somatic sensory and visual areas of the cortex project ipsilaterally, mainly to the large opiate receptor sparse areas of the striatum that we have termed the "matrix". Motor cortex projects bilaterally to both patch and matrix zones. Finally, we have identified a small area of frontal cortex, termed the prelimbic cortex, which projects primarily to the opiate receptor patches. These studies suggest a segregation of function within the striatum and also suggest that there are separate cortical channels to these distinct striatal compartments.

2. Identification of cortical transmitters. We have found that a large number of pyramidal neurons in cortical layers V and VI label with an antibody to glutaminase, an enzyme important in the synthesis of glutamate. This finding suggests that these neurons may use glutamate as a neurotransmitter. We predict that many cortical neurons that project to the spinal cord, brainstem, and thalamus use glutamate as a neurotransmitter. Fewer cells in the superficial layers label with this antibody, suggesting that many corticocortical cells could use a different, presently unidentified transmitter.

3. Chronic recording experiments. We have established a reliable method for single-unit recording in behaving rats. About 360 units have been recorded in somatic sensorimotor cortex during the forelimb task described above. We have

found that there are two parts of motor cortex in the rat. A caudal zone receives mainly cutaneous inputs and a cytoarchitectonically distinct rostral receives inputs from the muscles or joints. Thus, the rostral part of MI may be involved in the initiation of movements and in the control of the dynamic aspects of force output by the muscles. To identify the role of modulatory inputs on cortical discharge the locus coeruleus was stimulated during the bar pressing task. Initial data suggest that the locus coeruleus input enhances the activity-related discharge of some single units in AG₁ during movement, but has a direct inhibitory effect on others.

4. Intracortical circuitry. Three multipolar (local circuit) and three layer III pyramidal cells have been injected with HRP in cases where pyramidal tract neurons were also labeled by retrograde transport methods. These studies have shown that individual layer V multipolar neurons distribute their axons over a region 300-500 microns in diameter and contact numerous pyramidal tract neurons, although an individual neuron appears to make only a few contacts with any single neuron.

Significance to Biomedical Research and to the Program of the Institute.

Elucidation of the mechanisms of cortical information processing in the motor cortex, especially the role of the different afferent inputs and cell types in providing the cortical output, will provide a better basis for understanding normal and abnormal cortical function in all mammals, including humans.

Progress and Proposed Course of the Project:

This project will terminate because Dr. Donoghue will leave the laboratory July 1, 1984. This project will terminate with publication of a short communication on the activity of single neurons in motor cortex, a full-length publication on the same subject, a review article on rodent motor cortex and a report on corticostriatal projections.

Publications:

Donoghue, J.P., Wenthold, R.J. and Altschuler, R.A.: Localization of glutaminase-like and aspartate amino transferase-like immunoreactivity in neurons of cerebral cortex, J. Neurosci. in press, 1984.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 01335-14 SMRA

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of Schizophrenia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: R.J. Wyatt Chief APB, NIMH

Others: L.B. Bigelow Senior Investigator APB, NIMH
 D.R. Weinberger Senior Investigator APB, NIMH
 J.E. Kleinman Senior Investigator APB, NIMH
 W.J. Freed Senior Investigator APB, NIMH
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COOPERATING UNITS (if any)

University of California at Irvine, University of Finland,
 Harvard University School of Medicine, Johns Hopkins University School of Medicine,
 University of Minnesota School of Medicine, Laboratory of Preclinical Pharmacology,
 AFIP, LCNSS and LPP.

LAB/BRANCH

Adult Psychiatry Branch

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MAN-YEARS:

27

PROFESSIONAL:

25

OTHER:

2

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies performed within the Unit on Schizophrenia include studies employing regional cerebral blood flow and the BEAM technique. Post mortem studies examined brain morphology and neurochemistry. Of the psychopharmacology studies, investigations have been into the disorder of tardive dyskinesia, phenylacetic acid, dopamine, lithium carbonate and zimelidine. Pharmacogenetic studies have been performed to begin separating the relative influences of genetic and nongenetic variables on drug response. Biochemical studies have examined water regulation and calcitonin. Work continues on monoamine oxidase and alpha-adrenergic receptors. Investigations into the relationship of blinking to the schizophrenic syndrome continue as does research into the viral hypothesis of schizophrenia. Also, ongoing study of multiple personality disorder has progressed.

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J.M. Morihisa	Staff Psychiatrist	APB, NIMH
D.V. Jeste	Staff Psychiatrist	APB, NIMH
F.W. Putnam	Staff Psychiatrist	APB, NIMH
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C.A. Kaufmann	Senior Staff Fellow	APB, NIMH
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W.B. Lawson	Senior Staff Fellow	APB, NIMH
R.F. Zec	Senior Staff Fellow	APB, NIMH
A.C. Church	Senior Staff Fellow	APB, NIMH
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H. Yang	Visiting Pharmacologist	LPP, NIMH
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R. Zeigler	Faculty	Univ. of Minnesota School of Medicine
D. Asher	Senior Investigator	LCNSS, NINCCDS
E. Bernstein	Faculty	Dept. Psychology, America University
D. Ludlow	Senior Investigator	CDP, NINCCNS
T. Zahn	Senior Investigator	LPP, NIMH
R. Coppola	Senior Investigator	LPP, NIMH

Objectives

The long range goals of our research into the schizophrenic syndrome are to improve diagnostic abilities, to delineate etiologies and to develop optimum treatment methods. While attainment of these objectives remain well beyond today's grasp, our principle objective is to work towards this goal by performing basic and clinical research in a broad range of disciplines and psychiatric subspecialties. Our emphasis is in the direction of biological psychiatry, attempting to produce new knowledge, simultaneously synthesizing prior information in a multidisciplinary manner. The research of the Adult Psychiatry Branch pursues understanding of the schizophrenic syndrome through investigation of the psychological, biochemical, neuropathological, and anatomic aspects of this disease process.

Clinical Services

The clinical services for the research inpatients of the Adult Psychiatry Branch are provided under the overall supervision of the Clinical Director, Llewellyn B. Bigelow, M.D. Staff support comes from the William A. White Division of Saint Elizabeths Hospital, also under Dr. Bigelow's direction. There are three nursing units or wards, each with a capacity of 12 to 16 patients. The principal focus of the research in this branch is an effort to understand the biological causes of and to develop superior treatments for the major public health problems posed by chronic schizophrenia. It is the purpose of the clinical support services to provide first rate patient therapeutic care in a setting which also permits ethical and well-designed research. The patients, all of whom must be voluntary, are recruited from many sources. Some refer themselves, in other cases families contact the National Institutes of Health seeking assistance, and patients are often referred by several local hospitals who are aware of the treatment and research opportunities offered by our program.

Patients stay on the research units for up to two years while they participate in both research and rehabilitative programs. A full range of rehabilitative services is offered, led by the physician ward administrator and strongly supported by our excellent nursing staff and full time social workers, occupational and recreational therapists; ancillary programs are also available. Of particular note is the work experience program known as Industrial Therapy which permits patients to work at their own rate and at tasks suited to their clinical condition. No patient is charged for services received while enrolled as a research volunteer.

A typical patient stay in the William A. White Division would be something as follows: A four to six week period is planned for initial diagnostic studies and stabilization of the patient to the new environment. Therapeutic relationships are established and a detailed history is collected. Upon admission the patient is placed on coded medication only, usually similar to that which he was taking prior to program entry. This is to ensure that subsequent changes in medication may be performed "double blind" to minimize the effects of rater and patient bias in the measurement of treatment responses.

After this initial six week period has past, the patient will be switched blindly by coded medication to placebo medication. This period, lasting up to six weeks, is particularly important in that it permits the establishment of a diagnosis with the patient in a neuroleptic free state. This period also permits taking biological measurements such as spinal fluid, blood, and urine as well as performing other noninvasive procedures without contamination of results by high levels of strong medication. This drug free period is terminated if, in the opinion of the ward administrator and clinical director, such termination is wise and in the best interest of the patient or the program.

After completing the drug free interval, the patient is then placed on a fixed dose haloperidol regimen in order to establish behavioral and biological response in comparison to other patients. At the conclusion of six weeks of fixed dose haloperidol, there is a second drug free period lasting again up to six weeks during which time short term infusions are performed. These assess neuroendocrine status and provide data for other challenge studies. Assuming the patient has been shown to be somewhat responsive to neuroleptics, he is then placed again either on haloperidol or, if the response seemed less than optimal, on a different neuroleptic, usually mellaril or navane, and stabilized once again for optimal drug response. It should be noted at this time that no patient is enrolled in the program who is able, given conventional neuroleptic treatment, to lead a productive life outside the hospital

setting. Therefore, all patients in our program are either nonresponsive or only insufficiently responsive to known treatment.

After the two drug free treatment periods are completed, consideration is given to assigning the patient to an appropriate protocol, involving the addition of novel medication to his neuroleptic medications currently under study. These might include lithium carbonate, fenfluramine, clonidine, or bromocriptine. All, for a variety of theoretical reasons, show some promise as at least possible candidates as therapeutic agents in the treatment of chronic schizophrenia.

A major concern of the clinical section of this Branch is to obtain valid quantitative ratings of each patient's pathology. The backbone of this system is a daily rating by nurses using a scaled version of the psychiatric rating scale, modified by Dr. Bigelow. Since no rating scale, however well conceived, can produce data any better than individuals are trained to obtain, a major effort is focused on continued fine tuning of the rating process. This is accomplished through weekly meetings of the nursing staff on both day and evening shifts to address ongoing problems.

Additional rating measures are used, such as sleep observation and physicians' ratings of negative symptoms, as circumstances dictate. The ongoing program on tardive dyskinesia also utilizes nurse movement disorder ratings weekly or more frequently. We have on line computer terminals in each ward that can be used for the direct entry of rating data. Maintaining data in this way greatly increases reliability of ratings, enabling up-to-date monitoring of the ratings on each patient.

In order to oversee, monitor, and coordinate research and treatment efforts on each of the three units, the Clinical Director meets weekly with each individual ward administrator and holds weekly research rounds on each of the three units, alternating individual management and research issues. Potential conflicts between clinical and research needs can be discussed indepth during the individual meetings with more controversial or difficult issues referred for group discussion at weekly Rounds.

The format of the weekly Rounds involves all of the research staff assembling on one of the nursing units. Each patient is interviewed by a member of the senior staff in front of the group for a brief period. Opportunity is always given for questions from the patient as well as from the research staff at large. Although at first glance such a procedure might seem intimidating for patients, experience has shown that they appreciate the interest shown and opportunity to be heard by the entire research group, most of whom are familiar to them from intermittent daily interactions. After Rounds, which last about an hour, the research group assembles for a Post-Rounds Conference where each patient on all three units is reviewed briefly and any particularly difficult problems concerning research, or clinical issues posed by a patient's condition, are discussed by the group at large.

An additional function of the Post-Rounds Conference is to distribute, as equitably as possible amongst the many investigators of the Branch, permission for participation in specific protocols. There are so many protocols and ideas under development and investigation in the Branch that no one patient can participate in all of them. Major limiting factors include but are not limited to length of time it is appropriate for a patient to be free of neuroleptic medication, the degree of difficulty of cooperation required by particular protocols, and potential biochemical complex among protocols. For instance, many protocols require a minimum two-week time elapse on stable medication in order to

have neuroreceptors, theoretically at least, in a steady state. This is true particularly of challenge tests.

A current major effort is the central documentation of significant findings both of history and of current status of each patient. It is hoped within the next year to reduce this effort to a form which can be incorporated into an ongoing data base. One important aspect of this effort is the establishment of diagnoses late in the initial or second drug free period. The patient is given a structured interview. The interview is recorded on audio tape. At the conclusion of the interview, research diagnostic criteria and DSM III criteria forms are completed for each patient and a diagnosis derived. Additionally, the psychiatric rating scales and other specialized scales are completed and archived. Clinical data derived from each patient is considered the property of the entire research unit and not under the single purview of any investigator. The social worker for the Division is a skilled senior person with a special interest in family history. Family therapy is conducted regularly for those families where it is indicated if geographic obstacles can be overcome. Recreational activities are frequent with many outings to neighboring sites of interest. A recreational therapist comes in on weekends to lead activities.

In summary, the clinical activities of the Adult Psychiatry Branch have been structured to provide optimum therapeutic impact as well as a research setting for the study of the devastating syndrome of chronic schizophrenia. It is a supportive environment for investigators and patients from which it is hoped that new understanding and treatments will emerge.

Having given a general overview of our Clinical Services Program, the past reporting year has seen the initiation and completion of several psychopharmacological studies. Specifically, Dr. Bigelow and his team have begun therapeutic studies of verapamil. Data are being collected at this time.

Dr. Bigelow and his team, also, have completed the initial phase of our haloperidol dose response protocol. Preliminary findings indicate that a therapeutic response can be achieved at much lower dosages than are commonly given. These findings, if replicated consistently in larger populations, will have broad implications for the use of this drug.

Neuropsychiatry

Clinical Neurophysiology Laboratory

After four years of preparation, this year has seen the beginning of the operation of the neurophysiology lab. This lab is designed to investigate EEG and evoked potentials in psychiatric populations. The lab consists of EEG, evoked potentials equipment, high speed computer data processing and a computerized topographic mapping system that displays electrophysiologic data in condensed and summarized form. There are two laboratories that make up this facility. The first is an ongoing collaboration with Dr. Duffy using the Brain Electrical Activity Mapping (BEAM). The second is in collaboration with Dr. Rich Coppola. Three studies are being pursued in the collaboration with Dr. Coppola. The first investigates electrophysiologic abnormalities in childhood autism. In the second study, in which Dr. David Shore is also participating, a pilot study has been completed investigating electrophysiologic differences in Alzheimer's patients and normal controls. And the final study, with Dr. Andrei Iager is an investigation of the effects of vasopressin on electrophysiologic parameters of brain function in schizophrenia. Data are being collected and analyzed.

Also, Dr. Morihisa in conjunction with Columbia University has completed a preliminary investigation, using a computerized EEG method, to study the effects of electroconvulsive (ECT) therapy on patients with affective disorders. This study is the first topographic mapping of EEG changes in this population following consecutive ECT treatments. Data are being analyzed at this time.

BEAM

The development of computerized topographic mapping techniques in EEG and evoked potential research represents a further extension of the utility of electrophysiologic studies of schizophrenia. Until recently the massive amounts of data generated in EEG studies were impossible to resolve into succinct and easily assimilated pictures of brain function. Advances in solid-state electronics and pattern recognition software have rapidly expanded the capabilities of these topographic approaches. One such computer topographic method is Brain Electrical Activity Mapping (BEAM). This technique utilizes color maps to present EEG and evoked potential data in a condensed and summarized form. Further, this approach can develop, from these color maps, numerical descriptions that have the potential to discriminate schizophrenic patients from controls. Using these statistical tools we may further investigate electrophysiologic differences between various clinical populations. Encouraged by promising BEAM investigations of neurologic populations, Dr. John Morihisa and his colleagues have undertaken the systematic application of this technique in psychiatry.

This research has resulted in Dr. Morihisa receiving the A.E. Bennett Award for 1984. The award winning research was the first combined brain imaging investigation of schizophrenia to employ both BEAM and computerized axial tomography (CT) technology.

The results of this research demonstrated: (1) Increased frontal delta activity in patients with schizophrenia. (2) The ability to separate patients with schizophrenia from normal controls with summarized electrophysiologic data. (3) Electrophysiological differences over the frontal lobes of schizophrenic patients with frontal cortical atrophy compared to patients without frontal cortical atrophy. (4) A correlation between evoked potential amplitudes measured in the frontal regions and lateral cerebral ventricular size as measured by the VBR on CT scan. These findings are discussed in order. This original study is being expanded to investigate a greater number of subjects. Data are now being collected and will be presented in future Annual Reports.

Regional Cerebral Blood Flow

Of currently utilized techniques for functional brain imaging, Xenon¹³³ inhalation regional cerebral blood flow (rCBF) has unique advantages for the study of higher cortical function in psychiatry. This method utilizes Xenon¹³³ gas, a low energy gamma-ray emitting radioisotope, that is inhaled by the subject and used as a tracer of regional cerebral blood flow, a parameter closely coupled with cortical glucose metabolism. Since Xenon is a freely diffusible and inert gas, it exchanges readily between blood and tissue, yet fails to affect metabolic processes. If a tissue is first saturated with Xenon¹³³ and then allowed to desaturate, the rate of disappearance of the radioactivity is a direct function of the blood flow to the area in question. Since gamma-rays penetrate brain tissue and skull, external monitoring of the disappearance of radioactivity from a saturated brain is possible with this technique. By following the desaturation of a number of contiguous cortical regions, a regional distribution or map of blood flow, and by inference of cortical metabolism, can be studied.

In research performed by Drs. Berman, Weinberger, Morihisa and Zec, unmedicated schizophrenic patients have been studied serially with different cognitive tasks. This study is in preliminary stages, but early results are interesting. The study of the chronic schizophrenic patients shows a normal pattern of the frontal to occipital hyperfrontal gradient at rest. During the activation procedure, however, differences arise. Our results to date, while incomplete, suggest the following: (1) no significant differences in the ratio of frontal rCBF to parietal and occipital blood flow is apparent between the schizophrenic and control groups at rest; (2) when asked to perform the Wisconsin Card Sort Test (WCS), however, 40-50% of the chronic schizophrenics became hypofrontal compared with none of the control group. It is worth noting that the schizophrenic group makes errors on the WCS that are characteristic of frontal lobe patients, including perseverative errors and failure to maintain set.

Drs. Berman, Weinberger, Morihisa and Zec are continuing to pursue this finding by studying the cerebral metabolic response to this abstract reasoning task in a larger population of chronic schizophrenic patients and in a spectrum of patients with known frontal lobe abnormalities. Also, studies are being performed to investigate rCBF with subjects performing a vigilance and attention test, the continuous performance task to evaluate how specifically the findings are related to frontal cognitive demand.

The promise of functional brain imaging techniques such as rCBF is that brain-behavior relationships can be studied while the subject is alert and actively engaged in different sorts of psychological activation (e.g., cognitive, mood states, etc.). Biological concomitants of higher cognitive functions both in normal and pathological conditions may, thus, be investigated.

Neuropathologic Studies in Schizophrenia

Subsequent to the neuropathologic findings of decreased size of globus pallidus and hippocampus in schizophrenia (reported by Dr. Bernard Bogerts at the International Congress of Psychiatry, Vienna, 1983) and confirmed by a personal visit Dr. Stevens made to Dr. Bogert's Laboratory in the Vogt Institute of Pathology, Dusseldorf, West Germany in September 1983, Dr. Stevens and colleagues are now conducting immunocytochemical studies of peptides and aminergic putative transmitters in schizophrenic and control material.

Post Mortem Studies

Neurochemistry

The Adult Psychiatry Branch has been active in studying post mortem brains for neurochemical investigations. Dr. Kleinman has collected approximately 300 brains of patients with schizophrenia, affective disorders, heroin and alcohol addiction and normals. Twenty different brain regions have been used for measurements of catecholaminergic metabolites, indoleamines and metabolites, several neuroleptics, and several receptor or binding sites. The major effort has been in testing monoamine hypotheses of mental illness.

Dr. Kleinman and colleagues have continued to measure catecholamines and their metabolites in nucleus accumbens, hypothalamus and substantia nigra of schizophrenic patients, other psychotic disorders and normals.

Since last year, this work has continued in the following directions: (1) catecholamines (norepinephrine (NE) and dopamine (DA)); (2) indoleamines (serotonin, 5HT); (3) acetylcholine (ACH); and (4) viruses. These studies have been carried out on brains of patients with schizophrenia and non-schizophrenic subjects who have committed suicide. Also, reduced haloperidol has been measured in schizophrenic brain tissue as long as 70 days following the last dose.

Studies in the area of catecholamines are in progress and we plan to measure NE, DA and their metabolites in suicides and schizophrenic subjects in nucleus accumbens (n.a.), hypothalamus, cingulate and frontal cortex, amygdala, hippocampus and mammillary bodies. Previous positive findings include increased NE in n.a. of chronic paranoid schizophrenic subjects. In addition, α_2 receptor binding (NE receptor bindings) has been studied in schizophrenic subjects and suicides in pons using test tube receptor binding techniques and in locus coeruleus using autoradiography. Preliminary results in the former showed no differences while the later study is still in progress. A fourth group of studies are continuing with the measurement of dopamine sensitive adenylate cyclase activity (D-1 receptors) in n.a. and caudate of schizophrenic subjects, patients with Huntington's chorea and controls. Previous findings have shown increased D-1 receptors in n.a. and caudate of chronic schizophrenic patients. A fifth group of studies involves measurement of tritiated amphetamine binding and preliminary findings suggest decreased binding in n.a. of schizophrenic subjects. Finally, seasonal variations in catecholamines have been shown in catecholamines in n.a. and hypothalamus. Immunocytochemical localization of D-2 binding sites was attempted in lab animals for potential use in human studies.

Serotonin (5HT) and 5HIAA (5-hydroxy-indoleacetic acid, a major metabolite) have been measured using high pressure liquid chromatography (HPLC) in 14 brain regions of patients with schizophrenia and suicides. Positive findings include: (1) increased 5HT in globus pallidus and putamen and increased 5HIAA in occipital cortex of schizophrenic patients; (2) increased 5HT in globus pallidus and putamen of suicides and decreased 5HT in hypothalamus of suicides and increased 5HIAA in nucleus accumbens of suicides.

Studies of ACH have progressed on two fronts. First, muscarinic binding was measured in several brain regions of suicides with primarily negative results. Secondly, cell counts in ACH nuclei (nucleus basalis of Meynert) are being measured in demented schizophrenic subjects and non-demented schizophrenic subjects. Preliminary results suggest cell loss in the demented subjects relative to the non-demented subjects.

Viral studies have continued to test schizophrenic brain tissue for the presence of viruses using DNA hybridization techniques without obvious success. In addition, the possibility that schizophrenic brain tissue may contain a slow virus continues to be tested and some lab animals have developed neurological signs after injections with schizophrenic and control brain tissue.

Finally, diagnostic studies are underway using an NIMH developed post mortem scale titled DEAD (Diagnostic Evaluation After Death). In addition, a protocol has been approved for the premortem study of elderly patients at Saint Elizabeths Hospital whose brains might later be obtained.

Studies with live subjects have involved neuroendocrine and neurophysiological parameters in schizophrenic subjects. A number of hormones have been measured in schizophrenic subjects including prolactin, renin, angiotensin II, aldosterone, lutenizing hormone and FSH. Positive findings include correlations between prolactin and positive psychotic

symptoms in schizophrenic subjects with normal ventricles on CT scans. Similarly blink rates were reduced by neuroleptics in patients with normal ventricles, but were unchanged in large ventricle schizophrenic subjects. A study of neuroendocrine variables in water loaded schizophrenic subjects is in progress.

Also, Dr. Ko has been applying autoradiographic techniques to post mortem cryostat sections of the pons. He has examined alpha-2 adrenergic receptor numbers in the brains of schizophrenic, normal and depressed (suicide victims) patients. Tritated paraaminoclonidine (PAC) has been used as a ligand. Locus coreuleus receptor density is being compared in these three groups of specimens.

In short, post mortem studies appear to be a valid way to test or generate hypotheses involving mental illness. The Adult Psychiatry Branch plans to continue and expand this area of research.

Hippocampal Slices

Dr. Richard J. Wyatt and Mr. A. Paul Oliver have continued to perfect a method of long-term recording of cultured nerve cells. This developing technique has been tested with hippocampal slices with modest success. Current work is focused on developing a suitable cell culture technique for integration with the recording system.

Psychopharmacology

Neuroleptics and Tardive Dyskinesia

The development of the neuroleptic drugs, in the 1950's, revolutionized the treatment of psychosis. Unfortunately for many patients, however, these drugs have a very serious side effect - tardive dyskinesia. The still-unfolding story of neuroleptic-induced tardive dyskinesia has considerable relevance, not only clinically but also historically, philosophically, and legally for psychiatry as well as for the rest of medicine.

Neuroleptics have been found repeatedly to be the best available treatment for schizophrenia. Indeed, they are appropriately given a lion's share of credit for the dramatic drop in the number of public mental hospital patients from over 500,000 in 1955 to well under 200,000 by the late 1970's. But the history of neuroleptics and tardive dyskinesia shows how a valuable treatment modality may cause such serious side effects that these wonder drugs generate extremist calls for abandoning use altogether. Regrettably, the proven efficacy of neuroleptics in schizophrenia was viewed by some as a green light to use these drugs in large doses, for prolonged periods and for disorders in which the value of neuroleptics remained to be established. The progressive rise in the reported prevalence of tardive dyskinesia among neuroleptic-treated patients is, at least partly, attributable to the increasing use of these agents, often in high doses. According to some estimates, there may be about 100,000 patients with tardive dyskinesia in the United States alone. Yet banishing neuroleptics from psychiatric treatment because of the risk of tardive dyskinesia, would be throwing the baby out with the bath-water. Judicious use of these drugs for specific indications, in individualized and smallest effective doses is still required.

Because these drugs are so important and effective in the treatment of psychosis and, simultaneously, cause tardive dyskinesia in so many patients, increasing research resources are being devoted to its study. Research into tardive dyskinesia in the Adult Psychiatry Branch began in 1977 and continues to expand in various directions. Our tardive dyskinesia

work, spearheaded by Dr. Dilip Jeste and executed with a team approach of collaborating researchers from this Branch and other laboratories, studies the pharmacokinetic and psychopharmacological mechanisms of this disorder.

In the last reporting year, Dr. Jeste and his colleagues have investigated the pathogenesis of Tardive Dyskinesia from several directions. In one study examining elevated cerebrospinal fluid, norepinephrine was collected from 28 psychiatric (mostly schizophrenic) inpatients from Bombay, India. These included eight patients with tardive dyskinesia, five with spontaneous dyskinesia and 15 without dyskinesia. The samples were flown back to the National Institute of Mental Health where they were analyzed "blind" for concentrations of norepinephrine and several monoamine metabolites. Patients with tardive dyskinesia had significantly higher norepinephrine concentrations in the CSF as compared with the other two groups. The spontaneous dyskinesia group had significantly lower concentrations of homovanillic acid in the CSF. These results support the hypothesis of noradrenergic hyperactivity, rather than postsynaptic dopamine receptor supersensitivity, in tardive dyskinesia.

In other work, apomorphine and bromocriptine were investigated in relation to tardive dyskinesia. There is no satisfactory treatment for neuroleptic-induced tardive dyskinesia (TD). Although it is not known if it is safe to continue administration of neuroleptics to TD patients, a large proportion of schizophrenic patients with TD need neuroleptics in order to prevent psychotic relapse. It therefore seems important to test new therapeutic strategies against TD in schizophrenic patients who are receiving neuroleptics and still manifest the movement disorder. Catecholaminergic hyperactivity is believed to be one of the principle pathophysiologic mechanisms underlying TD. While some investigators attribute the putative catecholaminergic hyperactivity in TD to postsynaptic dopamine receptor supersensitivity, other biochemical processes such as noradrenergic overactivity may be more crucial in at least a proportion of TD patients. In any case, drugs that are presumed to lower catecholaminergic activity have received considerable attention as possible treatments for TD. Included in this category are low doses of apomorphine and bromocriptine.

Both apomorphine and bromocriptine are partial dopamine agonists and are presumed to have a biphasic effect on dopamine receptors: in large doses they stimulate postsynaptic excitatory dopamine receptors, while small doses act on inhibitory autoreceptors. This constitutes the basis for trying small doses of apomorphine and bromocriptine in TD patients. The spectrums of the actions of apomorphine and bromocriptine are, however, somewhat different. Although both drugs are potent D-2 receptor agonists, the effects on D-1 receptors are somewhat different. Apomorphine in low concentrations acts as an agonist, and in high concentrations as an antagonist at D-1, while bromocriptine is a weak partial agonist of D-1. There are also pharmacokinetic differences between the two drugs.

Most of the previous studies using these drugs in the treatment of TD have not been noticeably successful. In a recent review of literature on the treatment of TD, Dr. Jeste and colleagues found eight published studies of apomorphine and three with bromocriptine. About 30% of the TD patients treated had 50% or greater improvement with apomorphine and 20% patients with 50% or greater improvement with bromocriptine. There are at least two possible reasons for the relatively unsatisfactory results with apomorphine and bromocriptine: administration of doses that were relatively high, and use of these drugs in neuroleptic-free patients. Dr. Jeste, therefore, specifically designed a study to test the efficacy of very low doses of apomorphine and bromocriptine in medicated schizophrenic patients with TD. One of the TD patients also had multiple motor and vocal tics and other characteristics of neuroleptic-induced Gilles de la Tourette's syndrome.

On the basis of the supersensitivity hypothesis, one might expect that TD patients would have exaggerated clinical and biochemical response to dopamine agonists as compared with non-TD patients. To test this possibility, response of TD patients was compared with that of schizophrenic patients without TD, in terms of psychosis ratings and plasma concentrations of a dopamine metabolite, homovanillic acid (HVA). While plasma HVA is derived from both the brain and the periphery, some studies suggest that changes in central HVA may be reflected by parallel changes in plasma HVA.

Results showed that apomorphine produced no significant reduction in mean AIMS score. Only one patient had greater than 50% improvement in TD; this patient also experienced a moderate degree of sedation with the drug. Overall, apomorphine did not change BPRS scores significantly although it produced a significant drop in plasma HVA concentration 30 min after injection. TD and non-TD groups had similar responses in terms of BPRS scores and plasma HVA concentrations. Bromocriptine administration resulted in a 50% or greater reduction in AIMS score in three TD patients, including the one with tardive Tourette's syndrome. There was no noticeable sedation with bromocriptine. The drug lowered BPRS scores but had no appreciable effect on plasma HVA concentration. The drop in BPRS score after bromocriptine was significant for the entire group of 11 patients, but, because of smaller n's, failed to reach significance at 5% level for TD and non-TD subgroups separately. Placebo had no significant effects on BPRS scores, AIMS scores, or plasma HVA concentrations in TD and non-TD patients.

In a series of studies, Dr. Jeste and his team investigated the association of abnormal involuntary movements and negative symptoms of psychiatric disturbance. Spontaneously occurring movement disorders are frequently associated with psychiatric disturbances. There is no consistent pattern of psychopathology in patients with different types of movement disorders. Among the relatively common types of psychopathology seen in patients with Huntington's disease, Parkinson's disease, Wilson's disease and progressive supranuclear palsy is a constellation of psychomotor retardation and apathy, in the absence of cortical signs such as aphasia or apraxia. In view of the similarity of this symptom-picture to the "negative symptoms" of schizophrenia, we wished to explore the possible association of the negative symptoms with an iatrogenic movement disorder viz., neuroleptic-induced tardive dyskinesia (TD).

In one study, 47 RDC positive schizophrenic patients on our research wards were withdrawn from neuroleptics for at least four weeks. Nine of these patients were diagnosed as having persistent TD, eight as having intermittent TD, while the other 30 were free of dyskinesia. Psychopathology was rated on the Brief Psychiatric Rating Scale (BPRS), and dyskinesia on the Abnormal Involuntary Movement Scale (AIMS). These evaluations were performed "blind" each week. The mean of the BPRS scores during the last two drug-free weeks was taken as the index of neuroleptic-free psychopathology in the patient.

For each BPRS syndrome, the patients were divided into two groups, using the overall median score as the cut-off point. The persistent TD group was characterized by a significant overrepresentation of patients with high scores on depression ($p < 0.05$, Fishers exact probability test) and negative symptom ($p < 0.07$) subscales, and low scores on anxiety subscale ($p < 0.05$).

In another study, 36 inpatients were followed longitudinally for a mean of six months, with biweekly assessments. The patients received different doses of haloperidol during this time. The mean score of the persistent TD group on the negative symptom subscale of

BPRS was significantly greater than that of intermittent TD and non-TD groups ($p < 0.05$, Duncan's Multiple Range Test).

Results indicate an association between TD (especially persistent TD) and negative symptoms of schizophrenia (that include emotional withdrawal, motor retardation and blunted affect). The relatively low levels of anxiety in the persistent TD group during the drug-free period, are consistent with the greater severity of negative symptoms. There is an obvious overlap between negative symptoms and depressive symptoms. These studies are being continued using a scale form negative symptoms newly developed in this laboratory by Drs. Iager and Kirch. This scale appears to be more sensitive and specific than other similar scales that have been used previously.

Findings from these studies support the reported association between movement disorders and symptoms such as psychomotor retardation and apathy. A number of possible explanations can be considered to interpret such an association. These include a suggestion that specific lesions in basal ganglia could produce both a movement disorder and a specific type of psychopathology.

In related research Dr. Jeste and colleagues measured RBC choline and plasma choline concentrations in 27 chronic schizophrenic inpatients. Both the blood choline measures had a significant test-retest reliability in patients whose neuroleptic status remained unchanged over a month. RBC choline correlated inversely with current neuroleptic dose. Patients with a low ratio of RBC choline to plasma choline were on significantly higher doses of neuroleptics, and also had significantly greater scores on the hostility/suspiciousness subscale of the BRPS. No significant association was found between blood choline and tardive dyskinesia. One patient with tardive Tourette's syndrome had a high RBC choline concentration.

Although the tardive dyskinesia work is one of the most extensive aspects of our Branch's neuroleptic research, because of the pervasiveness of neuroleptic drugs in the treatment of schizophrenia, other neuroleptic studies have been performed. One example is the investigations into the pharmacogenetics of phenylethylamine and the ability of various neuroleptics to block phencyclidine (PCP)-induced behavioral stimulation in mice. Also, Dr. Ko has recently completed a study elucidating the lack of effect of neuroleptic pretreatment on MHPG response to clonidine, an α -2 receptor agonist.

Pharmacogenetics

Pharmacogenetics deals with significant hereditary variations in response to drugs. Such a definition is a balance between a too restrictive use of the term (referring only to hereditary conditions involving adverse reactions to drugs, such as glucose-6-phosphate dehydrogenase deficiency with abnormal response to aspirin), and too broad a definition of the term (dealing with any condition in which drug response is influenced by genetic factors). Clinical studies of pharmacogenetics are difficult because of variable contributions of hereditary and environmental factors to almost every instance of responsiveness to drugs. This fact applies no less to psychotropic drugs than to other types of chemical agents. Large-scale well-controlled, prospective studies of psychopharmacogenetics are very difficult to conduct because of practical and ethical considerations, as well as the problematic interpretation of results. While animal studies are easier to carry out, the interpretation of such data is hampered, also, by difficulties in separating the relative influences of genetic and nongenetic variables on drug response.

The development of recombinant inbred strains of animals has provided a unique approach to studying pharmacogenetics, including psychopharmacogenetics. This technique makes available, at the same time, animals from several generations: a pair of progenitor of parent strains (which are selected because of behavioral differences between the two, and each is then inbred for at least 20 generations), their reciprocal hybrids (called F-1 hybrids) and a number of recombinant inbred strains (so called because each has a variable combination of genes from the two parent strains, and each of these strains is inbred for at least 20 generations). Inbreeding is achieved by brother-sister mating and is expected to result in a progressively increasing degree of homozygosity. Inbreeding for 20 generations should produce almost 100% intra-strain homozygosity.

In a series of studies, a variety of neuroleptics were compared for their ability to block phencyclidine (PCP)-induced behavioral stimulation in mice. Methiothepin, fluphenazine, trifluoperazine, and chlorpromazine were highly effective in blocking phencyclidine-induced stimulation at doses that did not decrease spontaneous behavioral activity. Clozapine, thioridazine and haloperidol were moderately effective, while sulpiride, molindone, and pimozide were completely ineffective. The effectiveness of the drugs was found to be correlated with their ability to block tryptamine-induced seizures and with several other measures of antidopaminergic and antiserotonergic potency, it is concluded that a combination of antidopaminergic and antiserotonergic activity is important for blocking the stimulating effects of phencyclidine.

Widespread abuse of the drug phencyclidine (PCP), in recent years, has caused an increasing number of hospitalizations due to severe and sometimes violent toxic reactions. These adverse reactions frequently resemble psychotic episodes, involving violence, agitation and bizarre behavior and can last for several weeks. Frequently, therefore, some form of pharmacological treatment is indicated. The preferred form of treatment is controversial: some authors recommend benzodiazepines while others prefer neuroleptics. In animals, a number of studies agree that at least some of the effects of phencyclidine can be blocked by neuroleptics. There is little data, however, comparing various neuroleptics for their efficacy in blocking the effects of phencyclidine. Several studies have reported that both haloperidol and pimozide are effective blockers of various effects of phencyclidine. One study found that pimozide was somewhat less effective than haloperidol in blocking rotational behavior induced by phencyclidine. In addition, haloperidol has been found to be less effective than chlorpromazine and clozapine in blocking the locomotor stimulation in mice produced by phencyclidine. Another study in rats reported that butyrophenones, but not phenothiazines, antagonized some behavioral effects of phencyclidine. These findings suggested that there may be substantial differences between neuroleptics in their ability to block the various effects of phencyclidine. The purpose of the present study was to compare a variety of neuroleptics for their ability to block the stimulant effects of phencyclidine in mice.

To study these possible differential effects a total of 450 adult female Swiss-Webster mice were housed in groups of 4-8 and allowed free access to food and water. The animals were housed on a 12-hr light-dark cycle. Each mouse was used once only.

Phencyclidine HCl (1-(1-phenylcyclohexyl)piperidine HCl), was administered by intraperitoneal injection. In a previous study, this dose was found to produce 67% of the maximal degree of stimulation. Maximal stimulation was produced by 7.5 mg/kg.

Phencyclidine produced a pronounced stimulation of motor activity. The mean (\pm SEM) stimulation ratio for the phencyclidine-treated mice was 2.02 ± 0.15 , as compared with a mean stimulation ratio of 0.52 ± 0.04 for animals that did not receive phencyclidine.

Most of the neuroleptics that were tested blocked this effect of phencyclidine, in that they reduced the stimulation ratios as compared to those of mice treated with phencyclidine alone. In addition, most of the drugs reduced the activity of the animals prior to the administration of phencyclidine. The three drugs that did not block the effects of phencyclidine were molindone, pimozide, and sulpiride. To avoid the potential pitfall of missing a significant blocking effect of pimozide due to testing too few animals, or insufficiently large doses, 24 mice were tested at a very large dose (0.2 mg/kg). Even at this large dose, pimozide did not significantly decrease the stimulation ratios although there was a tendency in that direction. Smaller doses of pimozide and all dosages of molindone that were tested tended to enhance the effect of phencyclidine. Pimozide and sulpiride also did not decrease the activity of the animals prior to the administration of phencyclidine. In addition, the blocking effect of haloperidol, although statistically significant, was incomplete and was not dose-dependent.

These data confirm a number of previous reports that the behavioral effects of phencyclidine in animals can be attenuated by neuroleptics. In general, the best antagonists of phencyclidine (methiothepin, fluphenazine, and trifluoperazine) are very potent clinically (on a mg/kg basis) in schizophrenia, while several of the less potent antagonists (thioridazine, clozapine, molindone, and sulpiride) are also less potent clinically (on a mg/kg basis). Chlorpromazine, however, was very effective, even though it is not very potent clinically. Pimozide and haloperidol were relatively ineffective despite their great clinical potency. Pimozide was essentially ineffective and the blockade of the effects of phencyclidine that was produced by haloperidol was not dose-dependent and was never complete. This suggests that some property of neuroleptics other than their antidopaminergic activity contributes to their ability to antagonize phencyclidine-induced stimulation in mice.

The neuroleptics are thought to act clinically, in the treatment of schizophrenia, by blocking central dopamine receptors. Many neuroleptics, however, have substantial antiserotonergic activity as well. In this study the ability of neuroleptics to block the effects of phencyclidine was strongly correlated with their ability to block tryptamine-induced seizures, a presumed measure of antiserotonergic activity. The blocking activity was also correlated with the inhibition of spiroperidol and the binding of D-lysergic acid diethylamine (LSD) in the frontal cortex, both of which are measures of antiserotonergic activity. In addition, significant correlations were also obtained with several measures of antidopaminergic activity, such as inhibition of binding of neuroleptics and inhibition of dopamine agonist-induced stereotypy. Perhaps, therefore, phencyclidine has a combination of dopaminergic and serotonergic properties. Phencyclidine does not, however, interact directly with dopamine binding sites. It may be that either a particular form of antiserotonergic or antidopaminergic activity is capable of blocking the effects of phencyclidine or that a particular combination of antagonistic properties is effective.

In a related study by Dr. Freed, the stimulation of motor activity by phencyclidine was found to differ significantly in BALB/c and C57Bl/6By inbred strains of mice. Phencyclidine-induced stimulation was compared for these strains, their reciprocal F1 hybrids, and their recombinant inbred offspring. There were significant differences in responsivity among the strains, suggesting a genetic influence on the PCP response; however, the strains did not segregate into two distinct groupings, suggesting that this genetic influence was not carried

by a single gene. In addition, there was no relationship between the responsivity of these strains of mice to PCP and their previously-reported responses to amphetamine or scopolamine, which suggest that PCP-induced stimulation is not a simple cholinergic or amphetamine-like response.

In the final study reviewed in this section, Dr. Jeste and colleagues used inbred strains of mice to study the possible differential effects of d-amphetamine on acquisition of the conditioned avoidance response using a two-way shuttlebox paradigm. d-Amphetamine (5 mg/kg, i.p.) was administered prior to a test consisting of 100 trials, pairing light and sound as the discriminative stimuli, followed by an electric floor shock as the negative reinforcer. Naive male animals (n=about 8 from every strain) of two inbred progenitor strains (C57BL/6By or B6 and BALB/cBY or C), their reciprocal F1, hybrids (B6CF1 and CB6F1) and 7 recombinant inbred strains (CXBD, CXBE, CXBG, CXBH, CXBI, CXBJ and CXBK) were studied. d-Amphetamine caused significant disruption of shock avoidance in one progenitor strain (B6), but had no effect on the C strain. There was no significant effect on nonspecific motor activity (pretrial and intertrial crossings) suggesting that disruption in acquisition of avoidance behavior was not related to the presence of competing motor response (e.g., stereotypy). Data in F1 hybrids and recombinant inbred strains were analyzed using univariate and multivariate statistics. The results were consistent with polygenic influence on amphetamine-induced disruption or facilitation of acquisition of conditioned avoidance.

Phenylacetic Acid

Phenylacetic acid (PAA) is a major metabolite of phenylethylamine (PEA) in man. Because of PEA's behavioral similarities to amphetamine, it has been the focus of considerable research since the early 1960's. Recently, PEA excretion was reported to be elevated in some chronic paranoid schizophrenic patients as well as in a group of bipolar depressed patients with psychotic behavior. Because of PEA's possible importance in psychiatric disorders, future research on PEA will inevitably require consideration of its metabolism. We have previously reported a gas-chromatographic mass-fragmentographic method for the assay of PAA in urine. Unfortunately, due to its low concentration in plasma, CSF, and brain tissue the urine method, without major modifications, cannot be applied to these media. To deal with this, Dr. Karoum and associates have employed a highly reproducible method for assaying both free and conjugated PAA in human plasma and monkey CSF. With this method, a rapid and reliable mass fragmentographic technique, results showed that in humans approximately 45% of total plasma PAA is conjugated in contrast to approximately 60% in monkeys. Both free and conjugated PAA concentrations tend to be higher in monkeys than in humans. Plasma mean concentration of total PAA in humans and monkeys are, respectively, 459.1 and 838 ng/ml. Approximately 55% and 25% of total PAA in the CSF are conjugated in humans and monkeys, respectively. Total PAA mean concentrations in human and monkey CSF are 41.6 and 84.2 ng/ml. Because over 90% of total urine PAA in humans is conjugated, it is concluded that over 50% of urine phenylacetylglutamine may be derived from kidney conjugation of free plasma PAA and/or from the kidney's preferential filtration of conjugated PAA as contrasted with free PAA.

In other work investigating PAA excretion in schizophrenia and depression, urinary phenylacetic acid (PAA) excretion was found to be decreased in a group of chronic schizophrenic patients, particularly in a nonparanoid subtype. No significant change in PAA excretion was observed in a group of 21 unipolar depressed patients. Urinary PAA was studied following the administration of phenylethylamine, monoamine oxidase inhibitors, a dopa decarboxylase inhibitor, a low phenylalanine diet, and phenylalanine loads in several groups of psychiatric patients and normal volunteers. While phenylethylamine ingestion

increased urine PAA, inhibition of both phenylethylamine metabolism and synthesis failed to alter urine PAA. These studies suggest that urine PAA is primarily derived from phenylalanine transamination of pathways not involving monoamine oxidase or both. The observed decrease in PAA excretion in some schizophrenic patients may reflect an alteration in this pathway. The high phenylethylamine excretion previously reported in some chronic schizophrenic patients is not directly related to the observed low PAA excretion.

Dopamine Turnover in Depressed Patients

A dopamine theory of depression, which postulates a relative lack of dopamine in central synapses during depressive symptoms, has been formulated. Furthermore, antidepressants other than monoamine oxidase (MAO) inhibitors have been demonstrated to inhibit uptake of dopamine. On the other hand, dopamine has been suggested to have an important role in the switch process and in manic symptoms of bipolar affective disorders.

In animal models, antidepressant treatments have been reported to reduce dopamine autoreceptor sensitivity, which could consecutively lead to an increment in the intrasynaptic dopamine concentration. Furthermore, MAO inhibitors reduce the metabolic breakdown of dopamine similar to their effects on other biogenic monoamines. Data concerning effects of antidepressant treatments other than MAO inhibitors on indexes of dopamine metabolism in humans are scarce and inconclusive.

Dr. Karoum performed studies on the interrelationship between outputs of urinary dopamine, homovanillic acid (HVA), and dihydroxyphenylacetic acid (DOPAC) in depressed patients, and the effects of five antidepressant treatments on these indexes of dopamine metabolism.

The main findings of the study were: (1) excretion rates of urinary dopamine and its metabolites did not correlate highly with each other; (2) only lithium carbonate and clorgyline, the antidepressants with mood-stabilizing activity in bipolar patients, reduced the sum of the urinary outputs of dopamine and its major metabolites; (3) two depressed patients who became worse (agitated and/or delusional) while receiving desipramine and one who had a similar reaction to clorgyline had increased urinary outputs of HVA when receiving the drugs.

Electroconvulsive Treatment and Lithium Carbonate

The norepinephrine theory of affective disorders postulates that depression is associated with a reduction of the transmitter norepinephrine in critical synapses within the CNS. This theory has been partly based on one of the known pharmacologic actions of the tricyclic antidepressants. They reduce the reuptake of norepinephrine and serotonin into presynaptic nerve endings and consequently potentiate the effects of the released transmitter in the synapse. During long-term treatment in man, this reuptake inhibition of norepinephrine is maintained, but the total turnover of norepinephrine as reflected in urinary measures as well as the concentration of free 3-methoxy-4-hydroxyphenylglycol (MHPG) in plasma is reduced. These adaptive changes are probably secondary to a reduced firing rate of presynaptic norepinephrine neurons, as has been demonstrated in the rat, and they are reflected in the reduced whole-body norepinephrine turnover in depressed patients.

A similar sequence of events seems to take place during long-term treatment with clorgyline, a monoamine oxidase (MAO) type A inhibitor, even though its mechanism of action is distinct from that of the tricyclic antidepressants. Zimelidine, a relatively

specific serotonin reuptake inhibitor that appears to be an effective antidepressant, significantly reduces urinary MHPG output with a proportional decrease of whole-body norepinephrine turnover.

An important test of the norepinephrine theory of affective disorders would be if an antidepressant treatment could be shown not to affect the noradrenergic system. The fact that zimelidine, a serotonin reuptake inhibitor, administered over time had clear-cut noradrenergic effects suggested that the research be extended. Dr. Karoum, therefore, investigated whether two other antidepressant treatments, electroconvulsive treatment (ECT) and lithium carbonate, without clear primary noradrenergic effects, would influence production of norepinephrine and its metabolites. Furthermore, we wanted to continue our efforts to use antidepressant-induced alterations in urinary norepinephrine and metabolite outputs to "fingerprint" biochemical effects of these treatments in depressed patients. We were faced by the lack of direct data in humans documenting the extent of biochemical specificity, if any, of possibly distinct antidepressant treatments.

The main finding of the study was that both ECT and lithium carbonate had significant effects on the noradrenergic system in depressed patients. The treatments reduced whole-body norepinephrine turnover, even though the effect of ECT for the whole group did not reach statistical significance. Electroconvulsive treatment was found to reduce whole-body norepinephrine turnover significantly in the five patients who responded to the treatment.

Urinary Monoamine and Metabolite Output

Since the early 1970's, 3-methoxy-4-hydroxyphenylglycol (MHPG) output has been quantified to classify depressed patients. It has been suggested that patients with low MHPG output are particularly responsive to treatment with norepinephrine-reuptake-inhibiting antidepressants, whereas patients with high MHPG output apparently respond better to serotonin-reuptake-inhibiting antidepressants.

The rationale for measuring MHPG has been the posulated unique role of this metabolite in the urine to reflect norepinephrine production in the brain. Such a role has been contested by the demonstration of rapid transformation of intravenously infused MHPG into vanillylmandelic acid (VMA), which is subsequently excreted in urine. Furthermore, it was recently demonstrated that in depressed patients, the urinary excretion rates of norepinephrine and its major metabolites normetanephrine, MHPG, and VMA correlate highly with each other. This finding renders improbable that in drug-free and physically healthy depressed patients, any one metabolite of norepinephrine would yield unique information independent of the others. To further elucidate this idea, Dr. Karoum and colleagues investigated the relative reliability of repeated measurements of norepinephrine, normetanephrine, MHPG, and VMA in several samples obtained from drug-free depressed patients.

The main finding of the study was that the reliability of output measurements of urinary monoamines and their metabolites in well-controlled, drug-free depressed patients could be broken down into three groups: MHPG and VMA were fairly reliable on the basis of even a single measurement and quite robust on the basis of the average of more than one measurement; serotonin, HVA, and normetanephrine were slightly less reliable but provided reasonably reproducible results in repeated measurements; and finally, dopamine, norepinephrine, 5HIAA, and phenylethylamine varied so much that even by multiple measurements we could only define a range over which a given patient would fluctuate.

Zimelidine

The tricyclic antidepressants (TCAs) amitriptyline and clomipramine, which are relatively specific for 5-HT reuptake blockade *in vitro*, are demethylated *in vivo* to active metabolites which act predominantly on norepinephrine (NE) reuptake. Thus the actual drug effect in a therapeutic situation is not confined to a single transmitter system.

In contrast is zimelidine (ZIM), a bicyclic compound with antidepressant efficacy documented in controlled clinical trials. Not only is ZIM a relatively specific serotonin uptake inhibitor, both *in vitro* and *in vivo*, but its demethylated metabolite, norzimelidine (NZIM) is a far more potent 5-HT reuptake blocker which retains specificity. Thus, NZIM is three to ten times more potent than imipramine (IMI) as a 5-HT uptake inhibitor and has only one-tenth to one-twentieth the potency of desipramine (DMI) as a NE blocker. During treatment with ZIM, steady state plasma concentrations of NZIM are severalfold greater than those of ZIM and only NZIM levels correlate positively with the degree of neuronal serotonin uptake inhibition.

In a study investigating ZIM action, a relatively specific serotonin reuptake inhibitor, ZIM was administered to 12 hospitalized healthy young male volunteers. Chronic but not acute ZIM caused a modest (23%) but significant elevation of plasma norepinephrine (NE) measured in the standing but not in the supine position. The 24-hr urinary excretion of NE itself was unchanged on chronic drug, whereas "whole-body" NE turnover was reduced by one week of ZIM, as evidenced by lowered excretion rates (both individually and summed with NE) of the metabolites 3-methoxy-4-hydroxyphenylglycol (MHPG), normetanephrine (NM), and vanillylmandelic acid. Lack of effect of ZIM on the NM/MHPG excretion ratio (which is increased by desipramine) indicated that ZIM and its major metabolite, norzimelidine (NZIM) are not acting by NE reuptake blockade. These data are consistent with modulating serotonergic influence on the noradrenergic system. Reduction of NE turnover and increasing the efficiency of the NE neurotransmission may be a common pathway of all clinically effective antidepressant treatments.

Seasonal Variation in Central Dopamine Activity

In recent years hypotheses concerning the role of biological rhythms in human psychiatric conditions have usually been applied to affective disorders. These disorders often demonstrate a cyclical course and, in the case of some depressions, a diurnal variation in symptomatology. In fact, recent reports have shown that artificially lengthening the photoperiod (illuminated fraction of the day) with bright full-spectrum light may have therapeutic effects in some severe depressions. Despite evidence of regular variation (rhythmicity) in the behavior of some schizophrenic patients, the rhythmicity of this syndrome has received much less attention than that of affective disorders. Hence, regular variation of potentially relevant biological parameters of schizophrenia has not been extensively investigated.

To investigate these rhythms, Drs. Karson, Berman, Kleinman and Karoum performed two studies focused on the seasonal variation of central dopamine activity in patients with schizophrenia and normal controls. In the two investigations, data were grouped and analyzed by season (i.e., spring-summer vs. fall-winter). The first study concerned blink rate, a putative measure of central dopamine activity; the blink rate for patients with schizophrenia was significantly increased during the spring-summer period. In the second study concentrations of catecholamines and their metabolites were measured in the hypothalamus and nucleus accumbens of normal and schizophrenic subjects. Findings include

a reduced concentration of hypothalamic dopamine in normal controls and a reduced concentration of homovanillic acid in the nucleus accumbens of patients with schizophrenia, both during the spring-summer period.

Water Regulation

Patients with chronic schizophrenia often demonstrate polydipsia and secondary polyuria, sometimes with a concomitant syndrome of inappropriate antidiuretic hormone (SIADH). Water intoxication may subsequently occur, with hyponatremia, seizures, stupor, coma and even death. Other somatic consequences have recently been reported including projectile vomiting, malnutrition, cardiomegaly, edema, and urogenital dysfunctions such as enuresis, urinary incontinence, hydronephrosis and renal failure.

Recent studies of water regulation in the mentally ill have involved either case studies or small samples of patients that are not sufficient for epidemiological purposes. While large studies were performed over four decades ago, diagnostic and clinical practices at that time limit their interpretability.

Drs. Lawson, Karson and Bigelow, investigating the prevalence of polydipsia and polyuria, collecting urines from 62 chronic schizophrenic patients. Two to three consecutive 24 hour urines were collected during a period of complete medication withdrawal of at least two weeks duration or during periods of stable neuroleptic treatment.

Results showed that urine output for the normal controls and the two patient groups during medication withdrawal showed significant differences. Urine output for schizophrenic patients exceeded that of the normal controls and the nonschizophrenic patients. No significant difference was found between the normal controls and nonschizophrenic patients. Seven of the 35 medication-free patients with schizophrenia exceeded the highest output of the normal control group.

Calcitonin

The final piece of work in the psychopharmacology section concerns calcitonin. Calcitonin is a peptide hormone secreted by the C-cells of the thyroid gland. Its primary physiological effect is to decrease plasma calcium and phosphorus concentrations. This action of calcitonin is particularly pronounced whenever plasma calcium becomes elevated. Calcitonin was discovered in 1962, and it was found that perfusion of the thyroid-parathyroid complex with hypercalcemic solutions caused a lowering of plasma calcium concentrations. These results could not be explained in terms of decreased secretion of parathyroid hormone, which increases plasma calcium. Thus, it eventually became established that the thyroid secretes calcitonin, a hormone that decreases plasma calcium.

Calcitonin also has been found to inhibit eating. Drs. Beaurepaire and Freed have reported that calcitonin microinjections into several hypothalamic nuclei including the paraventricular hypothalamus, the supraoptic nucleus, and the perifornical area profoundly inhibit eating. The hypothalamus may therefore be the target organ for calcitonin in the regulation of eating behavior.

Synthetic salmon calcitonin is one of the most potent anorectic agents known; dosages of less than 10 micrograms per kilogram body weight markedly inhibit eating for 24 hours or more in rats and in monkeys. Calcitonin also inhibits eating when administered into the rat cerebral ventricles, in dosages at least 50-fold smaller than those required to inhibit eating

when administered peripherally. This suggests that the eating-suppressant effect of calcitonin is mediated by the central nervous system, and raises the possibility that calcitonin serves as an endogenous messenger that acts on the brain to inhibit eating behavior. The specific site of action of calcitonin within the brain is unknown. Although calcitonin binding sites are found in the rat hypothalamus, apparently calcitonin is not produced in the brain. It is, however, possible that calcitonin produced in the periphery or by the pituitary enters the brain in the hypothalamus, where it inhibits eating through an effect on hypothalamic neurons. Drs. Beaurepaire and Freed have determined, however, whether small amounts of calcitonin can inhibit eating when locally injected into various hypothalamic regions.

In animal studies, the sites where calcitonin produced the greatest inhibition of eating were the paraventricular hypothalamic nucleus (PVH), the perifornical area, and the supraoptic nucleus. A pronounced inhibition of eating, averaging more than 80%, was produced by all cannula sites throughout the PVH, from its anterior to its most posterior part. Dorsal to the PVH, the nucleus reuniens was also sensitive to calcitonin but the effect was less pronounced. Anteriorly, the bed nucleus of the stria terminalis and the nucleus accumbens responded to calcitonin, but also less markedly. Three sites which were about 0.5 mm ventro-lateral to the PVH also produced an average 67% decrease in eating. Although there could have been some diffusion of the calcitonin injected at this site to the PVH, it is also possible that the area of sensitivity extended to some areas immediately adjacent to the PVH. A decrease in eating (average 69%) was also produced by infusions into the ventromedial nucleus.

Several sites at the floor of the hypothalamus were sensitive to calcitonin. An average 89% decrease in eating was produced by infusions in the supraoptic nucleus. In two animals, infusions in the internal part of the optic tract produced an inhibition of eating. These infusions also, however, involved the retrochiasmatic supraoptic nucleus. Surrounding areas, such as the lateral hypothalamus, the medial forebrain bundle, and the preoptic area, were not responsive. There were some exceptions: an area above the optic chiasm, which could probably be described as the inferior part of the medial preoptic area, and more anteriorly, the ventral and lateral parts of the vertical limb of the diagonal band, responded to calcitonin, but more dorsal parts of these structures were not responsive. Large areas of the hypothalamus not involving specific nuclei, such as the anterior hypothalamic area, the preoptic area, and the lateral hypothalamic area did not produce a response. Calcitonin injections which decreased eating also produced decreases in general behavioral activity. Some animals showed turning behavior after injections, but this turning was not consistently in one direction. Dyskinetic or other abnormal movements were not observed.

The role of the hypothalamus in the regulation of feeding behavior is well known. Infusions of norepinephrine in the paraventricular nucleus, the perifornical area, the supraoptic nucleus, the ventromedial hypothalamus, the medial preoptic area, and the nucleus reuniens increase feeding. In the paraventricular nucleus, opiate infusions also increase feeding while cholecystokinin injections in the medial hypothalamus decrease eating behavior. A role for the nucleus accumbens in the regulation of feeding has also been proposed. The sites at which calcitonin influenced feeding behavior in the present study thus correspond closely with the locations at which infusions of other chemical substances, particularly norepinephrine, also influence eating behavior.

The Viral Hypothesis Of Schizophrenia

History is replete with examples suggestive of associations between outbreaks of infectious disorders and psychoses. Reports from ancient and prerenaisance times indicate coincidence of epidemic illnesses (e.g. plague) with dementia-like disorders. Others such as Benjamin Rush and Karl A. Menninger, also, connected psychosis with influenza outbreaks.

To complicate matters further, there are several examples of known viral diseases that are, at least initially, diagnostically similar to schizophrenia. The rare Russian tick-born encephalitis, endemic to the Yakut Republic of the USSR, is said to be, in its chronic form, indistinguishable from classical schizophrenia. Other, more common viral encephalitides are also either initially confused with schizophrenia or develop post-encephalitic dementia-like sequelae. It was a Russian investigator who first proposed that a virus, present in the body for a long time in a latent state, could produce symptoms of schizophrenia. Subsequent Russian literature, particularly in the late 1950's to 1960's, focused heavily on a viral etiology of schizophrenia.

Recent establishment of the existence of "slow viral infections" (viruses having an incubation period of years to decades prior to producing symptoms) have led a few Western researchers to propose an etiologic similarity between neurodegenerative disorders and schizophrenia. The first human slow virus disease discovered was Kuru, a neurological disorder endemic to New Guinea and transmitted by cannibalism. Kuru, however, is unlike schizophrenia because it results in massive brain degeneration and ultimately death. Other diseases, also thought to be of slow-virus origin, such as Creutzfeld-Jacob disease and perhaps even multiple sclerosis, have clearly associated neurodegenerative changes. In addition, some conventional viruses, such as measles, can also produce neurodegenerative disorders, such as subacute sclerosing panencephalitis, many years after the initial contact.

Indirect support for the viral hypothesis has come from epidemiological studies of schizophrenia. The seasonality of schizophrenic births, a peak in late winter and early spring that coincides with the peak incidence of some viral infections, such as measles and rubella, has been described. The uneven prevalence of schizophrenia throughout the world, also, is a pattern similar to the occurrence of some known viral diseases.

Further support for this hypothesis has grown out of the application of specific immunological techniques. Serum and CSF immunoglobulins, often elevated in viral diseases, have been found in some studies to be increased in schizophrenic patients. This finding is not, however, consistent and the type of immunoglobulin elevated varies. Further studies of viral specific antibodies support the possibility that these elevations are a consequence of viral infection. Increased serum antibody titers to herpes simplex type I virus were reported in one study, although not subsequently confirmed. In addition, an elevation of the ratio of CSF to serum IgG antibody titers has been reported for both measles and cytomegalic (CMV) virus.

In previous work in our laboratory, Dr. Janice Stevens found gliosis in limbic related structures in the brains of schizophrenic patients. In subsequent work with highly purified polyclonal antibody against cytomegatovirus, prepared by Dr. Robert Yolken of Johns Hopkins Hospital, Dr. Stevens was able to confirm earlier evidence of viral antigen in only one brain in schizophrenic and control patients. Dr. Stevens is now repeating these studies in frozen brain specimens and casting a wider net in the form of tissue of brain, CSF and serum of schizophrenic patients, in collaboration with Dr. Richard Zeigler. Also involved in these studies are Dr. Charles Kaufmann and Dr. David Asher (NINCDS, Bethesda, Maryland).

Viral Transfer Studies

Brains from guinea pig and primates, inoculated five years ago by Drs. Carlton Gadjusek and Joe Gibbs intracerebrally with homogenates from schizophrenic and control brains. From specimens collected by Drs. Dan Weinberger and Joel Kleinman, are being examined by Dr. Stevens with special techniques for gliosis and other pathologic changes. This work is being performed in collaboration with Dr. Kaufmann and Drs. John Langloss and V. Paresi at the Armed Forces Institute of Pathology.

Experimental Epilepsy

Dr. Stevens is continuing studies of chronic epilepsy in the rat with evaluation of effects of intracranial GABA'ergic agents on audiogenic seizures with Dr. Mike Iadarola and attempts to develop chronic epileptic focus with ferrous sulfate or aluminum hydroxide. Some success has been achieved with the aluminum hydroxide preparation in the amygdala producing a focal epilepsy. Dr. Stevens and colleagues plan to study the distribution of muscimol binding in the inferior colliculus of rats with audiogenic seizures.

Blinking

Since the cornerstone of neurochemical research in schizophrenia remains the dopamine hypothesis, the lack of an easily quantifiable and readily available clinical measure of central dopaminergic activity particularly hampers neurochemical research in this direction. Investigations of the concentrations of dopamine and its metabolites in the cerebrospinal fluid (CSF), or of hormonal indicators of central dopamine activity, such as prolactin, have failed to confirm that schizophrenic patients have elevated central dopaminergic activity. Even post-mortem studies, which show that increased dopamine receptors in schizophrenic brains are equivocal, indicate that this difference may be caused by neuroleptic therapy.

A promising tool in this area appears to be spontaneous eye blink rates. Decreased blinking in Parkinson's disease, a condition associated with decreased central dopaminergic activity, and increased blinking in schizophrenia, suggests that blink rates are positively correlated with central dopaminergic activity. Also, apomorphine, a dopamine agonist, increases blinking in monkeys. Therefore, blinking could be a useful monitor of the functional state of this neurotransmitter. Moreover, blinking is readily quantified and can be observed even in the most uncooperative patients.

Dr. Karson expanded two blink rate studies to include investigation of patients with hyperkinesias including Huntington's chorea, Tourettes syndrome and dystonias. Also, Dr. Karson is beginning the study of tears in schizophrenic patients.

Eye Movement Deprivation and Spontaneous Locomotor Activity in the Rat

Experiments have been completed and data analyzed concerning the effect of peripheral REM deprivation on locomotor activity in the rat. Previous studies in Dr. Steven's Oregon laboratory demonstrated disruption of the circadian cycle of amphetamine-induced running behavior in rats deprived of peripheral extraocular movement. The current report extends these findings to include spontaneous motor activity.

Multiple Personality DisorderClinical Phenomenology

The concluding entry into this section of the Annual Report pertains not to schizophrenia but to another disorder: multiple personality disorder syndrome. To investigate and rigorously document the clinical and physiological phenomena, Dr. Frank Putnam and colleagues have developed a 386-item questionnaire. This instrument focuses on the clinical presentation, diagnosis, clinical and family history, treatment and outcome. It was developed, piloted and distributed to psychiatrists and clinical psychologists treating cases of multiple personality disorder in the United States and Canada. Results from an analysis of the first 100 cases indicate that multiple personality disorder is a clinical syndrome with a core set of dissociative and depressive symptoms and behaviors. The most frequent clinical presentation is that of an atypical depression with mood lability and a lack of sustained vegetative signs. The patient population sampled was predominately female (92%) with an average age of 31 years. There was a high incidence of self-destructive behavior and violence directed towards others in this population. This group of patients retrospectively reported an extraordinarily high incidence of physical and sexual abuse in their childhoods. The degree of psychopathology, particularly self-destructive behaviors, was highly correlated with the numbers of different types of abuse retrospectively reported by the patients.

Dissociative Scale

A self-administered scale to measure the frequency and range of dissociative behaviors is under development in collaboration with Eve Bernstein, Department of Psychology, American University. The reliability coefficients of the scale are very good with a split-half Spearman Brown coefficient of $r=.95$, and a test/retest Pearson $r=.787$ ($p < .0001$). Populations sampled for reliability measures include chronic schizophrenic inpatients and university undergraduate students. Validity studies are underway with a population of patients meeting DSM-III criteria for multiple personality disorder. When the reliability/validity testing are completed the scale will be administered to a number of carefully defined psychiatric patient populations including: chronic schizophrenia, major affective illness, eating disorders, anxiety and phobic disorders and post-traumatic stress disorders in collaboration with researchers within the NIMH Intramural Research Program and several university research groups.

Childhood Dissociation Disorders

A number of converging lines of evidence indicate that the development of chronic dissociative symptomatology is closely linked to the experience of severe childhood trauma, particularly physical and sexual abuse. A list of symptoms and behaviors strongly suggestive of major dissociative reactions in children and adolescents has been compiled and distributed to child welfare workers in the Washington, Maryland and Virginia areas. A cohort of children displaying these behaviors has been identified and will be followed in a prospective study. Instruments used to assess behavior include the Connors Parent Checklist and the Achenbach Behavioral Inventory. An additional extensive case-collection questionnaire for distribution on a national scale is under development.

Electrophysiology

Current studies on the psychophysiology of dissociative states include measuring and mapping EEG and evoked potential changes among the alternate personalities of subjects meeting DSM-III criteria for multiple personality disorder. A group of psychodrama instructors, who have created imaginary alter personalities are serving as control subjects. The current focus of these studies, done in collaboration with Dr. John Morihisa, is the examination of shifts in hemispheric activity which occur with switches of alternate personalities, who vary in dominant handedness. These same subjects are also participating in Xenon inhalation cerebral blood flow measurements in collaboration with Dr. Daniel Weinberger.

Speech Studies

In collaboration with Dr. Christie Ludlow, of the NINCDS, voice spectral analysis studies, including test/retest at six month intervals, have been conducted on the alternate personalities of selected multiple personality disorder patients. Currently, an age and gender-matched control group of psychodrama instructors is undergoing testing. Initial data analysis indicate pronounced shifts in the format frequencies of the alternate personalities of the patient group which are not matched by the control group.

Autonomic Physiology

In collaboration with Dr. Theodore Zahn of the Laboratory of Psychology, galvanic skin response recordings and other autonomic measures were collected on the alternate personalities of 10 multiple personality disorder subjects and age and gender-matched simulating control subjects. The data are currently being analyzed.

Methods

Appropriate methods are employed and where possible, studies are performed in a double-blind design. Also whenever possible, sample sizes are large enough to allow for generalizable conclusions.

Significance to Biomedical Research and the Program of the Institute

Schizophrenia affects approximately one percent of the population, or about 2.2 million persons in this country. Given the number of individuals afflicted with this condition and the high cost to the nation, our research into potential etiologies, prevention and treatments of this disorder are highly significant.

Our research into the schizophrenias spans and connects a wide range of psychiatric subspecialties. Our biochemical studies are focused, primarily, on elucidating biological markers for the disease. But our Program throws an even wider net and includes research into other psychiatric disturbances such as the major affective disorders and multiple personality disorder syndrome.

Proposed Course

We plan to continue examining the schizophrenia syndrome from a multi-disciplinary perspective. To this end, we will continue to search biochemically for markers through the elucidation of abnormalities in the production and function of catecholamines, enzymes and

hormones. We plan to continue refining our ability to assess neuroanatomical and metabolic findings derived from such technological innovations as computerized axial tomography (CT) scans, cerebral blood flow and positron emission tomography scans. We plan to continue our etiological investigations into a possible viral component of the disorder. And in a manner that coherently connects this diverse body of research, we will continue to work towards a more productive typology of the schizophrenia syndrome and a greater understanding of the major psychiatric disorders of adulthood.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 01337-13 SMRA

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies on Drugs of Abuse

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	R.J. Wyatt	Chief	APB, NIMH
Others:	W.J. Freed	Senior Investigator	APB, NIMH
	E. Parker	Senior Investigator	NIAAA, ADAMHA
	S. Hashtroudi	Faculty	George Washington Univ. School of Medicine

COOPERATING UNITS (if any)

National Institute on Alcohol Abuse and Alcoholism
George Washington University

LAB/BRANCH

Adult Psychiatry Branch

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MAN-YEARS:

4

PROFESSIONAL:

4

OTHER:

0

CHECK APPROPRIATE BOX(ES)

(a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Over the past year, all studies of drugs of abuse have been inactive except for the conclusion of research to assess effects of alcohol consumption on memory function. Final results are reported in this section. This year's report, therefore, concludes the Adult Psychiatry Branch's project on drugs of abuse.

Objectives:

The objective of the Substance Abuse Program in the Adult Psychiatry Branch has been to formulate and investigate hypotheses concerned with the nature and action of pharmacological agents that are either classified as or can become, through misuse, drugs of abuse. Through research examining the mechanisms of action of these substances, we have come to better understand their use and the effects of their abuse.

AlcoholMemory

A major goal of research on alcohol and memory has been to identify the memory processes that are disrupted during intoxication. There is compelling evidence that failure to engage in elaborative processing is a critical source of alcohol-related memory impairment. Recent studies, however, suggest that certain forms of memory or certain memory processes are independent of elaboration. These processes are relatively unimpaired in amnesic patients, including alcoholic Korsakoff patients and might also be resistant to amnesia produced by acute alcohol intoxication. This study, the final study of the drug abuse project, by drawing on studies of the amnesic syndrome, examined the differential vulnerability of memory processes to alcohol amnesia.

Despite severe memory deficits in tasks requiring elaboration, amnesic patients show relatively normal retention in a variety of memory tasks. Early studies suggested that amnesics could acquire and retain motor skills and learn and perform maze problems. In a series of classic studies, researchers extended the domain of preserved memory processes to verbal materials. It was reported that amnesics did not differ from controls in identifying visually degraded words. Neither amnesic patients nor controls had been able to identify the degraded stimuli before being exposed to these words. After the study trials, however, the groups were indistinguishable in their identification performance. More recent evidence indicates that amnesics show considerable memory savings in reading inverted texts and in interpreting homophones according to their recently biased meanings.

To examine the differential vulnerability of memory processes to amnesia, Dr. Hashtroudi and her colleagues selected three different memory tasks: free recall, identification of visually degraded words, and recognition. Intoxicated and sober subjects studied the same list of words and participated in one of three retention tests. The free-recall test, which clearly requires elaborative processing, was included as a sensitive measure of acute alcohol amnesia. Impaired recall under alcohol should, therefore, provide a comparison for possibly preserved retention during intoxication.

The results of the recall and identification conditions suggest that memory tasks requiring elaboration or interitem integration are particularly susceptible to alcohol amnesia, whereas tasks relying on perceptual fluency are resistant to disruption by alcohol. These results suggest certain similarities between temporary alcohol-induced amnesia and chronic amnesias produced by various sources. This similarity, however, does not extend to the recognition results.

In agreement with previous research, alcohol induced a significant decrement in free recall. A free-recall test, of course, requires interitem integration and elaboration. Since other studies have shown that elaborative processes are disrupted under alcohol with the same dose used in this experiment, it seems reasonable to conclude that the observed recall

deficit reflects a disruption in the formation of interitem relations and elaborative processing during intoxication.

In contrast to the marked deficit in recall, retention as assessed by identification of degraded words was not impaired. Intoxicated subjects benefited to the same degree as sober controls from a single exposure to the item at study. The identification task has been characterized as a test of perceptual memory or "pure" familiarity. Regardless of the specific characterization, the identification task seems to be independent of elaborative processing. Preserved identification performance under alcohol suggests that alcohol has differential effects on perceptually based versus elaboratively based memory processes.

Methods

Appropriate methods have been employed and, where possible, studies have been performed in a double-blind design. Also, whenever possible, sample sizes have been large enough to allow for generalizable conclusions.

Significance to Biomedical Research and the Program of the Institute

Our concluding work in the drug abuse area has focused on the effects of alcohol consumption. The significance of performing research on substances becomes readily apparent when considering the pervasiveness of its misuse and the damage to the individual, family and society that its abuse brings.

Our work with alcohol has been highly significant, particularly in light of the well-known statistic that there are in excess of ten million alcohol abusers in this country. Even though we have relatively precise estimates on the extent of alcohol abuse, we have little definitive information on the effects of alcohol on memory. Thus, our work, beginning to differentiate those mental functions most severely hindered by alcohol consumption, is of importance to both the general population and the scientific community.

This terminates the Adult Psychiatry Branch's project on drugs of abuse.

Publications

Hashtroudi, S., Parker, E.S., DeLisi, L.E., Wyatt, R.J. and Mutter, S.A.: Intact retention in acute alcohol amnesia. Journal of Experimental Psychology 10: 156-163, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01338-06 SMRA

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of Aging

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	R.J. Wyatt	Chief	APB, NIMH
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	W.J. Freed	Senior Investigator	APB, NIMH
	D. Shore	Senior Staff Fellow	APB, NIMH
	A.C. Church	Senior Staff Fellow	APB, NIMH
	C.N. Karson	Staff Psychiatrist	APB, NIMH
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TOTAL MAN-YEARS:

16

PROFESSIONAL:

15

OTHER:

1

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Unit of Geriatric Psychiatry performs research into several aspects of the aging process. In the area of Alzheimer's disease, studies have been performed examining the roles played by aluminum and fluoride. Examining the differential effects of neuroleptic drugs on the elderly, diagnostic criteria for tardive dyskinesia have been further refined. Also, studies of cerebral ventricular size are being performed with Alzheimer's patients.

Another direction of the work of this unit is in the area of brain tissue grafting in Parkinson's disease. Transplantation of fetal and adrenal medulla tissue continues into the brains of parkinsonism model rats and monkeys. Histology techniques continue to be refined and inroads into questions of immunocompatibility are being pursued. And finally, in work also designed to eventually correct neural damage, research into nerve repair has continued, focused primarily on analysis of basic results of various nerve repair techniques.

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A. Seiger	Faculty	Karolinska, Institute, Sweden
L. Olson	Faculty	Karolinska, Institute, Sweden
B. Hoffer	Faculty	Univ. Colorado Medical Center

Objectives:

The research objectives of the Unit on Geriatric Psychiatry are to test existing hypotheses and create new hypotheses relating to the social, psychological, cognitive, physiological or affective changes that occur throughout the aging process. Further, it is the Unit's objective to perform research that illuminates the differences between normal aging and pathology, synthesizing work from specific disciplines as well as from interdisciplinary efforts.

Senile Dementia

Aluminum, Fluoride, and Alzheimer's Disease

The most common organic brain syndrome in the elderly is a degenerative brain disease called Alzheimer's disease or senile dementia of the Alzheimer's type. This illness is characterized by a progressive decline in memory and intellect, and a deterioration of social, occupational and communication skills. It is estimated that three to five percent of the United States population over age 65 is afflicted with this disease. Many more Americans will be afflicted as the average age of our population continues to rise. There is, presently, no effective treatment for Alzheimer's disease.

In recent years, patients with Alzheimer's disease have been found to have accumulations of aluminum in the hippocampus and cortex of the brain. These accumulations are localized within the nuclei of those nerve cells showing the neurofibrillary degenerative changes typical of Alzheimer's disease. This degeneration is most commonly seen in areas of the brain that are associated, generally, with memory and higher mental functions.

In earlier work investigating the significance of the relationship between aluminum accumulations and Alzheimer's disease, Dr. Shore and colleagues measured serum aluminum concentrations found in hospitalized patients of similar age and gender. The results tended to confirm that the increases in nerve cell nucleus aluminum reported in Alzheimer's disease are not the result of a generalized overload of this metal in biological fluids.

These findings, however, did not eliminate many of the questions concerning aluminum's role in the etiology and progression of Alzheimer's disease. If aluminum is a contributor to the degenerative brain changes in Alzheimer's disease, attempts to remove this metal from the body could have significant effects on the course of the illness. In this regard, the fluoride ion is of particular interest since elimination of aluminum by urine and feces is significantly increased by fluoride and aluminum retention in the body, reportedly, is decreased. A mutual reaction may occur between these ions in the body, resulting in the formation of an aluminum fluoride complex with the result that aluminum is not retained in the organism.

To examine this possibility further, Dr. Shore and colleagues initiated several preclinical studies investigating the aluminum-fluoride complexes. For several reasons, Dr. Shore focused on the use of fluoride to complex the aluminum which has already been absorbed, rather than trying to prevent the absorption of aluminum. One factor was interest in identifying patients early in the course of Alzheimer's disease and attempting to prevent the further progression of dementia. Earlier work showed that such patients do not have elevated concentrations of aluminum in blood or cerebrospinal fluid. Since most foods contain only 1.6 to 30 mg Al/kg, and normally only small amounts (less than 5%) of aluminum are absorbed, we have been more concerned with the potential toxicity of that aluminum already present in Alzheimer's patients. Such patients may have a "vulnerability" to aluminum neurotoxicity on the basis of genetic or viral factors or other inability to prevent aluminum from accumulating on DNA in neuronal nuclei. Results of the preclinical studies using small samples showed that fluoride treated animals tended to have lower aluminum concentrations in the brain.

Dr. Shore, also, is conducting a double-blind placebo-controlled study of the ability of fluoride to prevent the progression of early Alzheimer's in outpatients. The results of this study are expected in the next reporting year.

Neuroleptics

Tardive Dyskinesia

Neuroleptics constitute our most effective form of treatment for schizophrenia. Long-term administration of the antipsychotic agents, however, is fraught with the risk of inducing complications such as tardive dyskinesia that may be potentially irreversible. This danger is much greater in the elderly than in the young. The risk of tardive dyskinesia is high in both types of elderly patient populations: patients who have been diagnosed as having schizophrenia in early adulthood and who have continued receiving neuroleptics through old age; and elderly persons who, for the first time, develop psychosis and who are then placed on neuroleptic treatment.

Tardive dyskinesia may be defined as a syndrome consisting of abnormal, stereotyped involuntary movements, usually of the choreoathetoid type, affecting the mouth, face, limbs, and trunk, which occurs relatively late in the course of drug treatment. While tardive dyskinesia occurs following treatment with drugs other than neuroleptics, neuroleptics are by far the most common cause of iatrogenic tardive dyskinesia. To refine the process involved in prescribing these drugs, Drs. Jeste and Wyatt proposed a set of diagnostic criteria that covers the aspects of phenomenology, disease history, treatment response and differential diagnosis. These criteria are being applied to the examination, diagnosis and treatment of our research subjects.

Concerning the clinical implications, tardive dyskinesia is not only common in the elderly (with prevalence figures in excess of 40% for inpatients with a history of prolonged neuroleptic treatment), but also tends to be severe and persistent (the rate of persistence after neuroleptic withdrawal is greater than 50%). Although a number of therapeutic strategies have been tried in dyskinetic patients, there is as yet no satisfactory method for treating this disorder.

Our research continues to confirm our position that the use of neuroleptics in the elderly should be restricted to specific indications such as those outlined in the American Psychiatric Association Task Force on Tardive Dyskinesia. There is little justification for

prescribing neuroleptics as sedatives. Even relatively short-term administration of these drugs to geriatric patients carries a risk of producing persistent tardive dyskinesia. The following is a list of suggested guidelines that has been developed from our continuing investigations into the action of neuroleptic drugs and their relationship to tardive dyskinesia in an elderly population.

- 1) Neuroleptics should be prescribed only for well-justified indications, in smallest effective doses and for shortest possible periods. The dosage requirements for older patients are often smaller than the standard doses for younger adults given in the Physician's Desk Reference. The available data do not support a recommendation for drug-free periods to prevent tardive dyskinesia.
- 2) The risk of tardive dyskinesia should be discussed with the patients and their families.
- 3) Both the need for neuroleptics and the fact of having discussed the risk of tardive dyskinesia with patients and families should be documented.
- 4) Patients should be examined for the presence of dyskinesia before, and periodically after, starting neuroleptics. The findings should be documented.
- 5) Anticholinergic and antihistamine drugs should not be prescribed unless specifically indicated.
- 6) The need to continue neuroleptics should be regularly documented.

We are continuing to study the interaction of long-term neuroleptic treatment and the aging process in our patients.

Computed Axial Tomography

Over the past five years there has been a proliferation of research studies of schizophrenic patients evaluated by computed tomography (CT). This landmark radiological technique has revealed that some schizophrenic patients have CT scan findings suggestive of cerebral atrophy. The findings include enlarged cerebral ventricles, dilated cortical fissures and sulci, and possibly, reduced radiodensity of the cerebral parenchyma. Although negative studies have appeared, the majority of the controlled investigations have confirmed these findings.

For a variety of reasons, most investigators have concentrated on schizophrenic patients in the third and fourth decades of life. In fact, only one study has included patients over sixty years of age. The rationale for selecting primarily young patients is that since signs of cerebral atrophy are uncommon in this age group, subtle atrophic changes will be more readily appreciated. In elderly populations where CT findings consistent with cerebral atrophy are common, it would be more difficult to differentiate subtle pathology, possibly related to the schizophrenic illness, from the non-specific concomitants of normal aging.

But because the possibility of relating morphological change to neuropathology is of such interest to researchers of the aging process and the use of CT scan technology is so promising Dr. Weinberger and colleagues have been studying Alzheimer's patients. At this time are being collected and findings will be reported in next year's Annual Report.

Nerve Repair

It is considered axiomatic that complete severance of a peripheral nerve results in an absence of conduction across the gap, even if the nerve is repaired. Regardless of the method of repair, impulses initiated in the proximal stump of a severed nerve have not been recorded in the distal stump prior to restoration of the continuity of the fibers by regeneration. The purpose of our work is to determine if, and under what conditions, conduction can be obtained following reconnection of the stumps of a freshly transected peripheral nerve.

Recovery from peripheral nerve injury can be studied by a wide variety of techniques. These include nerve and muscle electrophysiology, clinical or functional tests, the pinch test, measurements of axonal transport, and histology. The most important criterion, however, is the degree of functional recovery.

Dr. de Medinaceli's work since the last reporting year has been focused, exclusively, on analyzing basic results of different techniques of nerve repair. The general purpose has been to demonstrate what makes cellular surgery better than current nerve repair techniques.

Three types of lesions were compared. These types are crush injury lesions, lesions produced by transection repaired by suture and transection lesions repaired by reconnection. Immunohistochemical techniques were applied to obtain a precise vision of the autoimmune responses. Although data are still being analyzed, preliminary results indicate that autoimmune response is much less severe after lesions are repaired by reconnection.

In a different series of experiments using another set of animals, analysis of the "dying back" of axons was performed by electron microscopy study. "Dying back" is a process of retrograde degeneration. Data are being analyzed at this time.

With a third group of animals, experiments using morphometric analyses were performed. Morphometric analyses are studies of number and calibre of fibers.

Results of this study will be reported in next year's Annual Report.

On one other group of animals behavioral studies were performed in order to correlate the findings in the previously cited studies with the functional results. Results of these experiments will not be available until all other study results are in and analyzed.

Parkinson's DiseaseBrain Grafts

Our work on tissue brain grafts has continued to expand since last year's Annual Report. As has been explained previously, rats with unilateral lesions of substantia nigra (SN) pars compacta, the area of the brain containing most dopamine-containing neurons, are a widely recognized animal model of Parkinson's disease. When given dopamine agonists such as apomorphine, these rats rotate in a direction contralateral to the lesion, presumably because of the development of supersensitive dopamine receptors in the striatum ipsilateral to the lesion. When grafts of embryonic SN are placed in the lateral ventricle, or into a transplant cavity adjacent to the striatum in animals with SN lesions, this rotational behavior has been shown to decrease. Histochemical examinations have shown that axons

from the grafts have grown into the striatum, and biochemical measurements indicate that dopamine concentrations are increased in areas of the striatum adjacent to the SN grafts.

In our original work, cited in previous reports, we have transplanted fetal rat tissue into the denervated substantia nigra of adult rats. We have been watching the progress of these rats to assess the prolonged survival and success of the transplants.

One obvious problem with this technique, however, both for basic research and possible clinical applications, is the requirement for fetal central nervous donor tissue. To circumvent this problem, we continue to seek other cells to substitute for the fetal tissue. We have found that the adrenal medulla contains some cells with similarities to some substantia nigra cells. In continuing studies investigating adult adrenal medulla grafts, Dr. Freed and his colleagues measured the concentration of catecholamines in adrenal medulla grafts as compared with the normal adrenal medulla. Results showed high, but extremely variable, concentrations of dopamine. In hosts with substantia nigra lesions, concentrations of dopamine in the adrenal medulla grafts were decreased. Substantia nigra grafts, however, tended to increase concentrations of epinephrine in the grafts, while norepinephrine and total catecholamine concentrations were not significantly affected. The conclusion from these results is that at least some adrenal medulla grafts contain concentrations of dopamine sufficient to account for their behavioral effects.

To further substantiate the results of our grafting research, we have been seeking more sophisticated means of histologic examination. It is a relatively simple matter to locate and identify several tissue fragments transplanted within the central nervous system. Intraventricular grafts stand out as tissue islands within a fluid space, and even intraparenchymal grafts generally display sharp borders and histological appearance distinct from the surrounding host brain.

In one group of rats receiving either of two types of catecholamine-containing tissues: 1) substantia nigra from 17-day gestational rat embryos and 2) adrenal medulla from 5-7 week old animals, fluorescence histochemical studies of the two types of graft tissue, using a glyoxylic acid technique revealed that both the substantia nigra and adrenal medulla grafts survived and contained specific fluorescence indicating the presence of catecholamines. The pattern and distribution, however, of catecholamine fluorescence in the two types of tissue differed markedly. Substantia nigra graft tissue was moderately fluorescent, containing cells resembling those in the substantia nigra pars compacta. The bulk of the catecholamine fluorescence associated with the substantia nigra grafts was usually, however, found not in the graft per se, but as fiber reinnervation of nearby regions of the host striatum.

Adrenal medulla grafts, in contrast, contained numerous brilliantly-fluorescent cells. These cells were similar in many cases to normal adrenal chromaffin cells, but a substantial proportion of these cells had either become elongated or developed short processes. These fibers did not reinnervate the host brain. Most adrenal medulla grafts contained regions of very tightly packed catecholamine-containing cells with intense specific fluorescence, suggesting the presence of high catecholamine concentrations. Around these areas, secretion and diffusion of catecholamines could be seen as a fluorescent "halo" or cloud in the adjoining tissues.

Concentrations of dopamine in punch samples from SN and adrenal medulla grafts measured by a gas-chromatography mass-fragmentography assay verified the results of the histochemical studies: SN grafts contained an average (\pm SEM) of 88 ± 33 ng of dopamine per mg of protein while adrenal medulla grafts contained a mean concentration of 796 ± 612 ng of dopamine per mg protein. The median concentration of dopamine in adrenal medulla grafts was 116 ng per/protein, but in many animals several-fold greater concentrations of dopamine were found.

In another study, Dr. Freed and colleagues investigated the report that ganglioside GM1 promotes reinnervation of the striatum by dopaminergic fibers following brain hemisection of the rat. That chronic ganglioside GM1 (10 or 50 mg/kg day for three weeks) would promote reinnervation of the dopamine-denervated striatum by embryonic substantia nigra grafts was studied. No enhancement of the ingrowth of fibers from the grafts was observed. It is concluded that under this circumstance, the growth of catecholaminergic fibers is restricted by factors other than the availability of ganglioside GM1.

Gangliosides, sialic acid-containing glycosphingolipids, are ubiquitous components of the mammalian cell membrane. They are incorporated into membranes with their carbohydrate residues facing outward, in contact with the extracellular fluid, and with the lipid moiety embedded in the cell membrane. These compounds are thought to play important roles in a variety of biological phenomena related to cell-cell interactions. Particularly high concentrations of gangliosides are found in the brain.

It has been recently reported that ganglioside GM1 stimulates the regeneration of dopaminergic neurons in the brain. Researchers have chronically administered GM1 ganglioside, by intraperitoneal injection, to rats which had received unilateral brain hemisections. This treatment partially restored striatal tyrosine hydroxylase activity and homovanillic acid concentrations ipsilaterally to the lesion. Consistent with these studies, others have reported that ganglioside GM1 promotes sprouting of motor neurons, process formation by neuronal, neuroblastoma, and PC12 cells and regeneration of central neurons. It has also been found that partial reinnervation of the corpus striatum in animals with SN lesions can be produced by grafts of embryonic substantia nigra (SN). In this experiment, therefore, Dr. Freed and colleagues investigated the effects of ganglioside GM1 on the reinnervation of the host brain produced by SN grafts in the lateral ventricle.

Results showed that all measures of reinnervation were found to be correlated with each other, except for the intensity of fluorescence in the reinnervated caudate. Thus, correlation coefficients for mean and highest score for the reinnervated area versus depth of fiber penetration and depth of the fluorescence intensity gradient were all statistically significant (all r_s greater than 0.4, $p < 0.03$).

There were also no significant differences in the properties of the grafts themselves that were measured. There was a tendency, however, for the brightest fluorescence in the grafts to be greater in the ganglioside-treated animals. This trend did not reach statistical significance ($p=0.08$). There was also a non-significant trend for the score reflecting maximum amount of the graft that was innervated to be greater in the ganglioside-treated animals.

One of the primary obstacles to treatment of Parkinson's disease patients by grafting procedures is the limited effectiveness of the grafts, both in terms of their limited behavioral efficacy and in terms of the amount of the host brain innervation produced by a single graft. One of the purposes of this experiment was to find methods of increasing the

amount of host brain innervation produced by a SN graft. No enhancement of the reinnervation of the host brain was produced by ganglioside GM1 treatment and thus ganglioside GM1 probably cannot be used to stimulate the outgrowth of fibers from SN grafts.

In addition to the continuing studies with rats, Dr. Freed and his team are assessing the effects of tissue brain grafts in rhesus monkeys and developing new testing methods on rat and monkey transplants. To this end, Dr. Patel continues to employ her procedure for the dissociation and implantation of adult adrenal medullary and fetal substantia nigra cells into adult rat brain. In the next years, the procedural methods developed will be used to test the behavioral effects of dissociated cell implants on unilaterally 6-hydroxydopamine substantia nigra lesioned rats. Similar methods with be utilized for experiments of fetal substantia nigra dissociated cell implants in monkeys.

In addition to improving survival rate and extending the functional effects of dissociated cell implants, Dr. Patel will also be undertaking ultrastructural studies of implanted adrenal medulla and substantia nigra tissue pieces as well as dissociated cell implants and their connectivity with the host brain. This will involve the tracing methods (HRP-WGA Histochemistry), standard electron microscope studies and methods for distinguishing the implanted cells in the host (i.e., immunocytochemistry and ³H-Thymidine labeling). These experiments will be the first ultrastructural characterization of implants capable of producing changes in lesion induced behavior.

Another major project to be continued will be the study of the implantation of rat fetal spinal cord into adult rat host spinal cord in both injured and uninjured animals. Dr. Patel has already developed an overlay technique in which fetal spinal cord can be introduced onto the pial surface of an adult host. The fetal spinal cord will then incorporate itself into the adult host whether or not the host spinal cord had been injured. Dr. Patel has demonstrated the survival and differentiation of such implants in adult host animals for up to one year. This type of implantation has potential for repair of injured spinal cord in that it is relatively noninvasive (i.e., the host spinal cord is not directly traumatized in implantation). Thus any spared function in an injured host cord would be presumably unaffected by the implant. The implant could then act as a tissue bridge for lesioned host axons without affecting intact axons. In the next few years this technique will be studied in injured rat host spinal cord to see if the implant is beneficial in functional recovery. Studies will be made behaviorally, and morphologically at the light and electron microscopic levels. The tracing technique for connectivity may be utilized also.

To conclude this section on brain grafts, embryonic brain cell transplantation has now been applied to a number of neuronal systems. In some experiments, brain grafts have been found to develop appropriate connections with the host brain. In others, brain grafts have been found to alter the behavior of the host animal. Recently, however, brain grafts have been shown to produce appropriate behavioral alterations associated with a reinnervation of the host brain.

This affords an ideal opportunity to correct lesion-induced behavioral deficits with brain tissue grafting. By placing small fragments of immature brain tissue in direct contact with the host striatum, the distance to be traversed by growing fibers is minimized and the development of fiber connectivity with the host brain is presumably facilitated. As this technique improves, questions of applicability to humans arise. Drs. Freed and Wyatt and their colleagues have, therefore, developed criteria to guide human application of brain tissue transplantation techniques.

The most difficult issue concerning embryonic brain tissue transplantation for neurological disorders is the problem of when and under what circumstances a procedure should be used clinically. Therefore a proposed set of research goals, completion of which would facilitate clinical trials of intracerebral tissue transplantation for the treatment of Parkinson's disease, has been devised. Fulfillment of these criteria is suggested only to provide sufficient evidence that the procedure would be likely to provide benefit to humans if the surgical procedure is successful. Emphatically, these criteria do not obviate legal and moral issues regarding the procedures, nor do they bear on the technical aspects of the surgical procedure per se.

- 1) Optimal Efficacy of a Single Graft. It would not be possible to systematically study all of the possible factors that might influence growth of the grafts. Thus, a reasonable effort to investigate some of the most promising parameters, such as grafts age and size, and a few hormonal and chemical factors, should be made.
- 2) Anatomical Distribution of Grafts. Whether or not the effects of a single graft can be substantially increased by various manipulations, a means of further distributing the graft tissue should be found. Thus, either through the use of a number of small grafts or individual dispersed cells, distribution of the area of influence of the graft tissue throughout most of the striatum should be achieved.
- 3) Minimal Behavioral Effect in the Rat. Accomplishment of the first two objectives should permit the production of more substantial behavioral effects in the rat. We suggest that the rat with unilateral SN lesions should be employed to test any prospective procedure using methods strictly analogous to those under consideration for humans. The following results in rats should be obtained: (a) Apomorphine-induced rotation should be substantially and consistently reduced so that a statistically significant reduction can be repeatedly demonstrated with small numbers of animals. (b) Other behaviors, such as sensory neglect, aphagia, or spontaneous locomotor asymmetry should also be favorably influenced, and (c) These results should be replicated in at least two independent laboratories using live tissue controls.
- 4) Scaling Factors. It will be important to have a basis for predicting the number and distribution of grafts that will be required for application to the human brain. The amount of reinnervation produced by the grafts might increase in proportion to the size of the brain or might remain constant irrespective of brain size. Thus the amount of reinnervation, degree of effect, or sphere of influence of a single graft should be measured in species of several sizes.
- 5) Immunology. Unless autografts can be used, the immunological privilege of the brain will undoubtedly play an important role in the success of the procedure. To prevent unforeseen immunological difficulties, it will be important to have verified the presence of immunological privilege for the circumstance relevant to the proposed clinical procedure. One particular unanswered question is whether the primate brain has immunological privilege.
- 6) Consistent Success in Primates. Brain grafts should also be tested in primates, and should be shown to survive consistently without evidence of ongoing deterioration. The procedure employed should be analogous to that to be used in humans.

7) Behavioral Effect in a Higher Order Species. The last criterion for human application is suggested to be a behaviorally-successful application of the prospective procedure to a larger and higher mammalian species. In an animal such as a cat, dog, or sub-human primate with SN lesions, symptoms analogous to those found in Parkinson's disease should be favorably influenced by a procedure strictly analogous to that proposed for humans. Major side effects or effects suggestive of substantial lesions should not appear following the surgery. And, the animals should survive for at least six months to one year following the transplantation procedure.

At the present time, catecholaminergic brain grafting procedures have been demonstrated to produce favorable functional effects in the rat. There are, however, several substantial obstacles, primarily involving the degree of effect and brain size, in translation of these procedures to humans. Fulfillment of the suggested criteria would, in our opinion, provide sufficient evidence that procedures for brain tissue transplantation in the lesioned nigrostriatal system are sufficiently well developed so that if they were performed in humans there would be a reasonable likelihood of clinical benefit.

Methods

Appropriate methods are employed and, where possible, studies are performed in a double-blind design. Also, whenever possible, sample sizes are large enough to allow for generalizable conclusions.

Significance to Biomedical Research and the Program of the Institute

The significance of our research into the problems of the aging process are best seen in the areas of Parkinson's disease, senile dementia of the Alzheimer's type, tardive dyskinesia, nerve repair and cerebral atrophy. In light of the shift in our nation's demographics towards an older population, increased understanding of these disease processes, neurological findings, and new methods of treatment are critically needed.

For example, Parkinson's disease, manifested primarily by abnormalities of movement and posture, is characterized by dopaminergic neuronal loss and gliosis in the brain. Current therapeutic approaches to Parkinson's disease involve administration of the drug L-dopa, a precursor of dopamine and dopamine-like agents. Despite some dramatic improvements, such therapeutic regimens are frequently not completely effective, or are associated with severe side effects. Many of these difficulties may result from, among other possibilities, the absence of the physiological mechanisms which normally regulate neurotransmitter release from dopaminergic terminals. Our work grafting dopamine-producing cells into the brains of Parkinson model animals attempts to circumvent this problem by developing a technique that would allow a previously damaged brain to begin reproducing the necessary dopamine. In developing this line of investigation intensive study has been generated internationally, leading to the first grafting operation in humans in Sweden, and since our last Annual Report work in this vein has proliferated among a variety of national and international laboratories.

Our examinations of the possible mechanisms involved in Alzheimer's disease are equally significant to the scientific community and the general population. Dementia is the major neuropsychiatric disorder of old age. According to figures issued by the National Center for Health Statistics, organic brain syndromes afflict 58% of the more than one million Americans in nursing homes. Many senile dementia patients are housed in other chronic-care facilities such as state mental hospitals and Veterans Administration hospitals.

More than half of the patients over age 65 in state and county mental hospitals also carry the diagnosis of chronic organic brain syndrome or senile dementia.

Senile dementia of the Alzheimer's type can be defined as progressive, age-related, chronic cognitive dysfunction. A number of hypotheses have been promulgated to explain the origins of the disease. One that has attracted much attention in recent years postulates an increased amount of aluminum in the brains of Alzheimer's patients. Our work into the possible role of aluminum, as well as our investigations of various potential drug treatments, is timely and needed research, and has continued to provide new data throughout this reporting year.

Our work into the prevention and treatment of tardive dyskinesia, also, is of vital importance to both the medical, legal and lay populations. Tardive dyskinesia is the most serious complication of long-term neuroleptic therapy. What was initially thought to be a rare clinical curiosity has become a significant public health hazard.

Typically, tardive dyskinesia occurs after years of neuroleptic administration, but we have shown that older patients are at increased risk even with short treatment schedules. The syndrome consists of abnormal involuntary movements of the mouth and face, extremities, and trunk. The pathophysiology of tardive dyskinesia is not precisely understood and there is no satisfactory treatment. Our award winning investigations into this disorder have generated both significant findings as well as new research directions in many other laboratories.

Proposed Course

We plan to continue our work into the prevention and treatment of Parkinson's disease, senile dementia of the Alzheimer's type, tardive dyskinesia, nerve repair and cerebral atrophy in the elderly. In our Parkinson's disease research our grafting work is expanding to include dopamine-producing tissue grafts into the brains of monkeys. In our Alzheimer's disease research we will be examining further the effects of sodium fluoride on the amelioration of the disease's symptoms as well as experimenting with potential pharmacological treatments. In our tardive dyskinesia work, we will be examining the potential of various enzymes as biological markers to determine which patients are at highest risk to develop the disorder. Our nerve repair research is continuing with refined means of quantification. By determining those most at risk, we should then be better able to develop treatment modalities that both control the patient's psychosis while reducing the risk that the patient will develop tardive dyskinesia.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01505-11 SMRP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neurotransmitter Dynamics: Chlordecone

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: O. Gandolfi Guest Researcher SMRP NIMH

Other: E. Costa Lab Chief SMRP NIMH

COOPERATING UNITS (if any)

J.-S. Hong, Lab. Behav. Neurol. Toxicol., NIEHS, Research Triangle Park, North Carolina

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Molecular Neurobiology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MAN-YEARS:

1.2

PROFESSIONAL:

1.1

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Adult male rats receiving a single i.p. injection of kepone (80 mg/kg) exhibit tremors within a few hours after the injection. Since kepone-elicited tremors are relieved by injections of muscarinic receptor blockers, we measured acetylcholine turnover in various brain structures. We failed to detect evidence for an involvement of cholinergic presynaptic mechanisms in kepone toxicity. Kepone inhibits the turnover rate of GABA in striatum. Moreover, we found that kepone down-regulated 5HT1 receptors and increased the turnover of serotonin in hippocampus and striatum. These results suggest the possibility that kepone decreases GABAergic tone indirectly by an increase of serotonergic firing thereby increase cholinergic tone in striatum, causing tremors.

Proposed Course:

This project has been prepared for publication and terminated.

Publication:

Gandolfi, O., Cheney, D.L., Hong, J.-S., and Costa, E.: On the neurotoxicity of chlordecone: A role for γ -aminobutyric acid and serotonin. Brain Res., in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 01506-10 SMRP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Narcotic Analgesics and the Regulation of Catecholamine Neurons

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	H. Kageyama	Guest Researcher	SMRP	NIMH
Others:	A. Guidotti	Section Chief	SMRP	NIMH
	P. Panula	Visiting Fellow	SMRP	NIMH
	E. Costa	Lab Chief	SMRP	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

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SECTION

Neuroendocrinology

INSTITUTE AND LOCATION

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TOTAL MAN-YEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The role of opiate receptors located on catecholaminergic cells was studied using primary cultures of adrenal chromaffin cells. These cells contain opiate receptors in measurable amounts. Stimulation of these receptors with agonists decreases the release of catecholamines elicited by nicotine. This effect is stereospecific and is reverted by naloxone and diprenorphine. One of the most potent opiate peptides is met-enkephalin-Arg6-Phe7. Probably this peptide coexists with enkephalin in the splanchnic nerve. Upon release it may function as a second chemical signal modulating cholinergic synapses by reducing the number of acetylcholine receptors available.

Project Description:

Enkephalin-like peptides coexist in association with other neurotransmitters in many axons, including the cholinergic axons of the splanchnic and the catecholaminergic chromaffin cells of adrenal medulla. From primary cultures of the latter they are released in association with catecholamines. It is hypothesized that these enkephalin-like peptides when released together with another transmitter act as a secondary chemical signal modulating the action of the primary transmitter which mediates neuronal communication. Our objective was to study the modulatory role of opiate peptides on the acetylcholine (ACh)-induced catecholamine release from primary cultures of bovine adrenal medullary cells. These cells possess high affinity stereospecific opiate binding sites; the addition of opiate receptor agonists to these cells inhibits the ACh-induced release of catecholamines, but not the release of catecholamines elicited by KCl or Ca^{++} ionophores. Thus the primary culture of adrenal chromaffin cells is an ideal model to study the molecular mechanisms whereby opioid peptides modulate nicotinic receptor activation that releases catecholamine from medullary cells.

Results

Various opiate receptor agonists with specific affinities for μ , δ , σ and K receptors have been compared for their ability to bind to adrenal membranes and for their potency to inhibit the ACh-induced release of catecholamines from chromaffin cells. Etorphine, β -endorphin, met-enk-Arg⁰-Phe¹ and the synthetic peptide D-Ala², Me Phe³, Met(O)-ol-enkephalin inhibited the acetylcholine-induced release of catecholamines with an IC_{50} varying from 10^{-7} to 1×10^{-6} M. The effect was stereospecific because levorphanol ($IC_{50} = 7.5 \times 10^{-7}$ M) was approximately 2 orders of magnitude more potent than dextrorphan. Morphine (μ receptor agonist), D-Ala²-D-Leu⁵-enkephalin (δ receptor agonist), ethylketazocine (K receptor agonist) and N-allylnormetazocine (σ receptor agonist) were at least 100-1000 times less potent than etorphine. Diprenorphine ($IC_{50} = 5 \times 10^{-7}$ M) and naloxone ($IC_{50} = 10^{-6}$ M) antagonized the effect of etorphine. High affinity, saturable and stereospecific binding sites for ³H-etorphine, ³H-dihydromorphine, ³H-D-Ala²-D-Leu⁵-enkephalin, ³H-ethylketazocine and ³H-N-allylnormetazocine, ³H-diprenorphine and ³H-naloxone were detected in chromaffin cell membranes and in membranes obtained from adrenal medulla homogenates. However the number of binding sites for ³H-etorphine and ³H-diprenorphine was 10 to 70 times higher than the number of sites measured with the other ³H ligands. The ranking order of potency of these compounds for the displacement of ³H-etorphine binding correlates ($r=0.96$) with the ranking order of potency of the same compounds for the inhibition of ACh-induced catecholamine release. These data suggest that a stereoselective opiate receptor (different from the classical μ , δ , K or σ receptor) with high affinity for etorphine, diprenorphine, β -endorphin and met-enk-Arg⁰-Phe¹ modulates the function of the nicotinic receptor in adrenal chromaffin cells. Adrenal cells contain receptors for muscimol and benzodiazepines. Their function in catecholamine and opiate peptide release is now being investigated.

Proposed Course

We intend to study the molecular mechanisms whereby the stimulation of opiate receptors produce a decrease of acetylcholine-induced release of catecholamine from adrenal medulla cells. We intend to develop a ligand capable of labeling

nicotinic receptors in nervous tissues, and using this ligand, characterize the possible consequences of opiate receptor occupancy on acetylcholine recognition sites. Presently a peptide obtained from Bungarus multicinctus venom appears to be a good candidate as marker for nicotinic receptors in adrenal medulla.

Conclusions

The opiate receptors are present in membranes of adrenal chromaffin cells. Activation of these receptors causes a non-competitive inhibition of the release of catecholamines elicited by the stimulation of nicotinic receptors. From our data, it can be inferred that when the met-enkephalin-like material is released from terminals of splanchnic nerves, it modulates the release of catecholamines induced by the stimulation of nicotinic receptors elicited by the concomitant neurally mediated release of acetylcholine. Alternative modulation of adrenal nicotinic receptor can be achieved by opiate-like peptides from blood (i.e. β -endorphin) or from the adrenal cells themselves. The adrenal medulla contains several types of opioid-like peptides; including met- and leu-enkephalin-like peptides, dynorphin 1-13, peptide E, met-enk-Arg⁶-Phe⁷, BAM 12P, BAM 20P and BAM 22P. Interestingly met-enk-Arg⁶-Phe⁷, a peptide present in high concentrations in adrenal medulla, and in the splanchnic nerve terminals (Panula, this lab) is one of the most potent opiates tested. It could be discussed whether met-enk-Arg⁶-Phe⁷ acts directly or after it is converted to met-enk. Our data provide clear evidence that met-enk-Arg⁶-Phe⁷ is an opiate agonist on its own right because met-enkephalin and DADLE (a stable analogue of enkephalin) are two to three orders of magnitude less potent than the heptapeptide. We propose that met-enk-Arg⁶-Phe⁷ is the putative endogenous opiate agonist which modulates the function of nicotinic receptors in adrenal medulla.

The interrelations between the opiate agonists and the nicotine-induced catecholamine secretion are relevant to the missions of the NIMH in many ways. The present study establishes a model to investigate how opiate receptor stimulation modulates the receptivity by chromaffin cells of incoming chemical signals. On a more general ground these studies may uncover the biological principle that regulates the functional interaction between a primary transmitter and a coexisting neuropeptide in nerve axon terminals.

Publications:

Costa, E., Guidotti, A., Hanbauer, I., and Saiani, L.: Modulation of nicotinic receptor function by opiate recognition sites highly selective for Met-enkephalin-Arg⁶-Phe⁷. Fed. Proc. 42: 2946-2952, 1983.

Kageyama et al. (J. Neurosci. Meth.) (Z01 MH 01574-02 SMRP).

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 01509-14 SMRP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Psychopharmacological Studies of Acetylcholine Turnover: Behavior

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	W. Blaker	Staff Fellow	SMRP	NIMH
Other:	E. Costa	Lab Chief	SMRP	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Molecular Neurobiology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MAN-YEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The behavioral and biochemical effects of neuropeptides and GABA agonist muscimol were studied by comparing the acetylcholine turnover rate (TR-ACh) in the rat hippocampus and hippocampal regions with extinction of a food reinforced lever press response after intraseptal injection of such compounds. We have shown that muscimol decreases the hippocampal TR-ACh and increases extinction responding in a dose-dependent manner. Intraseptal beta-endorphin, which decreases the hippocampal TR-ACh through an activation of septal GABAergic interneurons, also increases extinction responding. On the other hand, intraseptal substance P, which decreases the hippocampal TR-ACh in a manner unrelated to septal GABAergic mechanisms, fails to increase extinction responding. The TR-ACh in various hippocampal regions after intraseptal injection of muscimol and substance P was also studied. Muscimol decreases the TR-ACh only in the ventral hippocampus, whereas substance P decreases it only in the dorsal hippocampus. We hypothesize that a lowering in the cholinergic input to the ventral hippocampus is capable of increasing extinction responding, whereas a decrease in the input to the dorsal hippocampus is without such an effect. Hence, the cholinergic projections to the two hippocampal areas are modulated by different transmitter systems and have different physiological functions.

Studies were also performed to characterize the GABAergic control of the substantia inominata-cortical cholinergic pathway in the rat. It is known that activation of GABA receptors in the substantia inominata by microinjection of muscimol decreases the TR-ACh in the cortex. To test the hypothesis that such control may be tonic, in separate experiments bicuculline (a GABA antagonist) was injected into the substantia inominata and the major GABAergic input to the region was destroyed by lesioning the nucleus accumbens with kainic acid. Neither treatment altered the TR-ACh in the cortex. This indicates that the GABAergic control of this cholinergic projection is similar to that in the septal-hippocampal pathway, which also lacks a tonic GABAergic control.

1069

Project Description:

The present studies were undertaken to compare the biochemical and behavioral effects of the activation of septal neurotransmitter and neuropeptide systems on the rat septal hippocampal pathway. This was accomplished by correlating pharmacologically induced decreases in the turnover rate of acetylcholine in the hippocampus with changes in extinction of a food-reinforced lever-press response.

Numerous studies in the literature point to the involvement of the cholinergic septal hippocampal system in response inhibition, and thus extinction of a food reinforced lever press response was used as a behavioral indicator of proper functioning of this system. The turnover rate of acetylcholine in the hippocampus was used as a biochemical indicator of the cholinergic activity of septal projections to the hippocampus. In such a procedure, phosphoryl(^3H)choline was infused via the tail vein and the incorporation of label into choline and acetylcholine was determined using gas chromatography-mass fragmentography. From the choline and acetylcholine curves representing the change with time of the incorporation of the label, the fractional rate for acetylcholine efflux was determined. The fractional rate constant multiplied by the steady state concentration of acetylcholine yielded the turnover rate of acetylcholine.

The experimental protocol called for rats to be implanted with chronic septal cannulae and then trained on a continuous reinforcement scheduled over several days. The animals were then injected with various amounts of the appropriate drug via the septal cannulae and 20 minutes later challenged with extinction of the learned response for 10 minutes. This was immediately followed by the infusion of phosphoryl(^3H)choline via the tail vein for the turnover rate determination.

We have previously shown that intraseptal injection of the GABA agonist muscimol simultaneously and dose-dependently decreases the turnover rate of hippocampal acetylcholine and increases responding during extinction. This is in keeping with the histochemical evidence of a substantial GABAergic innervation of the septal nuclei. Also from previous studies, it has been shown that septal beta-endorphin can decrease the turnover rate of hippocampal acetylcholine through an activation of GABAergic interneurons. Conversely, septal substance P decreases this parameter independently from GABAergic mechanisms. Since these two neuropeptides have different modes of action, they were used in the present study to further explore the regulation of the septal-hippocampal cholinergic system.

We found that intraseptal beta-endorphin produced an increase in extinction responding at doses known to decrease the turnover rate of hippocampal acetylcholine. However, intraseptal substance P produced no behavioral effect in this paradigm. We studied whether the behavioral difference observed in the effect of intraseptal muscimol and substance P despite an apparently similar action on the turnover rate of hippocampal acetylcholine was due to the selective action of septal neurotransmitter systems on specific hippocampal cholinergic projections. The turnover rate of acetylcholine was analyzed in four hippocampal segments after the intraseptal injection of muscimol and substance P. Substance P decreased the turnover rate of acetylcholine in only the two dorsal segments, while muscimol decreased it only in the most ventral segment. This finding indicates that only the activity of those cholinergic neurons projecting to the ventral hippocampus is important in mediating the increased responding during extinction. Also, the

modulation of septal cholinergic axons projecting to different areas of the hippocampus has a high degree of pharmacological differentiation that may be of therapeutic use.

In another study, we tested the hypothesis which has recently been put forward that a causative factor in the degeneration of cholinergic projections to the neocortex found in Alzheimer's disease may be the initial loss of inhibitory GABAergic input to these cells, leading to their overstimulation and eventual death. A key assumption in this hypothesis is that the GABAergic inhibition of these cholinergic neurons is tonic. We reduced the GABAergic input to cells in the substantia inominata of rats (the origin of the cortical cholinergic projections) in two ways: a) acute treatment by microinjection of the GABA antagonist bicuculline into the substantia inominata, b) chronic treatment by lesioning the nucleus accumbens (the source of a major GABAergic input to the substantia inominata) with kainic acid. Neither treatment altered the turnover rate of cortical acetylcholine, making the above hypothesis unlikely.

This project is of high significance to biomedical research and to the NIMH program because it focusses on the role of septal hippocampal and septal cortical cholinergic pathways of memory because it is pertinent to keep in mind that presenile dementia is currently believed to be due to a functional and anatomical deficit.

Future course. We are developing methods to study memory acquisition and retention using more methods. In fact, the type of memory evaluated with this technology seem to be more responsive to hippocampal modulation. Moreover, methods will be developed to measure with isotopically-labeled acetylcholine turnover rate. Correlation between acetylcholine turnover changes in various hippocampal regions and memory acquisition, consolidation and retention will be evaluated.

Publication:

Blaker, W.D., Peruzzi, G., and Costa, E.: Behavioral and biochemical differentiation of specific projections in the septal-hippocampal cholinergic pathway of the rat. Proc. Natl. Acad. Sci. USA 81: 1880-1882, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
 Z01 MH 01514-12 SMRP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Trans-synaptic Control of Protein Synthesis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	H. Kageyama	Guest Researcher	SMRP	NIMH
Others:	A. Guidotti	Section Chief	SMRP	NIMH
	F. Tang	Guest Researcher	SMRP	NIMH
	T.T. Quach	Visiting Associate	SMRP	NIMH
	J.P. Schwartz	Research Chemist	SMRP	NIMH
	E. Costa	Lab Chief	SMRP	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Neuroendocrinology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MAN-YEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The addition of 8-Br-cAMP to primary culture of adrenal chromaffin cells, produces an induction of TH and a two- to three-fold increase in synthesis of enkephalin-like immunoreactive material (ELM). TH and ELM induction is preceded by an activation of cytosol cAPK and by an increase in nuclear protein phosphorylation. The induction of TH, the increase of ELM and the increase of nuclear phosphorylation require that the assembly of microtubular proteins be functional. Anti-microtubular drugs such as colchicine and vinblastine (10-9M) can block the TH and ELM induction elicited by 8-Br-cAMP when the drugs are added less than 15 hours after 8-Br-cAMP. Since colchicine, added with cAMP, also prevents the increase in nuclear phosphorylation, it is possible that the assembly of microtubular proteins might be operative in the intracellular translocation and nuclear uptake of catalytic subunits of cAPK activated by the addition of 8-Br-cAMP. In addition, these data support the view that an increase in nuclear protein phosphorylation is an essential step in the mediating the acceleration of mRNA synthesis and the subsequent increase in TH and ELM synthesis elicited by 8-Br-cAMP.

Project Description:

Our objective was to study the molecular mechanisms whereby transsynaptic stimuli induce new synthesis of specific proteins in chromaffin cells of adrenal medulla. In previous studies, we had reported that in chromaffin cells of rat adrenal medulla, the sequence of molecular events whereby transsynaptic mechanisms regulate the expression of tyrosine hydroxylase (TH) genes includes an increase in the cAMP/cGMP concentration ratio, an activation of cAMP-dependent protein kinase (PK) in cytosol, and the translocation of the low molecular weight catalytic subunit of this protein kinase from the cytosol to the subcellular particles. The PK of nuclei is not regulated by cAMP but increases during transsynaptic induction of TH because the cAPK catalytic subunits translocate from cytosol to the nucleus. Thus, activation and translocation of PK, triggered by the initial increase of cAMP, acts as a long range messenger for the transsynaptic expression of the genetic coding for TH.

Recently, it has been reported that two or more neuroactive substances coexist in the same axon terminals with the primary transmitter. They are coreleased with the latter and when released function as cotransmitters modulating the gain of the primary transmitter response at the receptor. Adrenal chromaffin cells are a typical example of this coexistence. Opiate peptides and catecholamines coexist and are released together during splanchnic nerve stimulation. Whether the opiates function as modulators of the catecholamines in periphery is not yet established; however cotransmitter function can be suggested for the opioid that coexists with acetylcholine in the splanchnic nerve.

In relationship with this coexistence, one wonders whether in the adrenal cells the synthesis of opiate peptides undergoes long term adaptive changes similar to those reported for TH. Indirectly this question imposes one to explore whether the genes that control synthesis of coexisting neuromodulators are operating under a common regulatory process. Until recently, interest in cotransmitter peptides had been focused primarily on synthesis and processing and not on the regulation of gene expression. However, by taking advantage of the development of recombinant DNA technology and in situ hybridization histochemistry, a better understanding of the regulation of gene coding for these modulators will be forthcoming. In our study we decided to use an in vitro system, primary cultures of cow adrenal medulla cells. These cells were used as a model to evaluate the ability of 8-Br-cyclic AMP (8-Br-cAMP) to induce TH an enkephalin-like immunoreactive material (ELM) and to study the role of cAPK in this induction. This cell culture maintains a constant level of cyclic nucleotides, catecholamines, ELM and related enzyme activities for about four weeks.

Exposure of the cells to 8-Br-cAMP produces 48 hrs later, a dose related longlasting increase in TH and ELM activity; 8-Br-cGMP fails to modify TH and ELM. The increase in TH activity caused by 8-Br-cAMP is due to an increase of the V_{max} and in the number of enzyme molecules; the increase in ELM is due to an elevation of high and low MW opiate peptides and is preceded by an increase of proenkephalin mRNA as determined by Drs. Tang, Quach and Schwartz (our lab) using complementary DNA. Both increases are preceded by an activation of cytosol cAPK associated with a decrease of the total cytosol cAPK. A sustained increase in nuclear phosphorylation begins 8 to 12 hrs after 8-Br-cAMP application. The delayed increase in TH and ELM activity induced by 8-Br-cAMP is blocked by actinomycin D, cycloheximide,

colchicine and vinblastine. This reduction of TH and ELM induction elicited by colchicine and vinblastine (10^{-9} M) is observed only when the inhibitors of the microtubular protein polymerization were added 4 to 12 hrs after the addition of 8-Br-cAMP, the inducing stimulus. The addition of colchicine 15 hrs after 8-Br-cAMP fails to inhibit TH or ELM activity. This blockade is associated with an inhibition of the increase in nuclear phosphorylation, but is not associated with an inhibition of protein synthesis. The increase of endogenous cAMP and the induction of TH were also produced by cholera toxin. These results suggest that the concomitant increase of TH and ELM elicited by 8-Br-cAMP is mediated by the translocation of cAPK subunits from cytosol to the nuclei and that this translocation requires the function of the microtubular network.

Since adrenergic mechanisms and opiate peptides have been implicated in the etiology of affective disorders, an understanding of the molecular nature of the regulation of the biosynthesis of catecholamines and enkephalins may lead to a better understanding of the synaptic defects that may be operative in the etiology of mental diseases. In addition, the translocation of cAPK subunits from the cytosol to the nuclei may operate as a basic mechanism in memory and/or learning.

In future studies, we plan to explore how the increase in nuclear phosphorylation regulates the expression of the gene coding for the induction of TH and ELM.

Publication:

Costa, E., Guidotti, A., Hanbauer, I., Kageyama, H., Kataoka, P., Panula, P., Quach, T.T., and Schwartz, J.P.: Adrenal medulla: Regulation of biosynthesis and secretion of catecholamines and enkephalins. In Usdin, E. (Ed.): Catecholamines, Vol. A: Basic and Peripheral Mechanisms. New York, Alan R. Liss, Inc., 1984, pp. 153-161.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 01516-11 SMRP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biochemical Pharmacology of Minor Tranquilizers

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: A. Novelli Guest Researcher SMRP NIMH

Other: A. Guidotti Section Chief SMRP NIMH

COOPERATING UNITS (if any)

Shionogi Research Laboratory, Japan

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Neuroendocrinology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MAN-YEARS:

1.2

PROFESSIONAL:

0.7

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Behavioral and biochemical studies have shown that benzodiazepine (BZD) action is mediated through GABAergic synapses. By virtue of such an interaction, the supramolecular organization of the GABA receptors could include several recognition sites for specific chemical signals. Among others, two separate binding sites (one for 3H-GABA and one for 3H-diazepam) were isolated by differential solubilization from rat brain homogenates with Triton X-100. Photo labeled benzodiazepine recognition sites were further purified by preparative SDS gel electrophoresis and reverse phase HPLC. By this technique a 1000-fold purification was obtained. Purification of BZD recognition sites may help to understand functional aspects of the supramolecular organization of the GABA receptor system.

Project Description:

Recent evidence from this laboratory has suggested that benzodiazepines may inhibit convulsions through a primary action on GABAergic transmission. Successively it was shown that practically all the pharmacological actions of benzodiazepines are mediated through GABAergic mechanisms. This interaction depends on the modulation of the number of GABA recognition sites when benzodiazepine (BZD) recognition sites are occupied by specific ligands. By studying the molecular structure of the benzodiazepine recognition site we hope to obtain a better understanding of the molecular interaction that are operative.

To study this problem we initiated studies devoted to the solubilization and partial purification from rat brain cortex homogenates of ^3H - γ -aminobutyric acid (GABA) and ^3H -diazepam recognition sites.

Results

Isolation and purification of BZD recognition sites was obtained by photo labeling the BZD receptor with ^3H -flunitrazepam. Purification of the photolabeled receptor was achieved by preparative SDS gel electrophoresis followed by reverse phase HPLC. After HPLC chromatography the purity of the material was checked by two dimensional polyacrylamide gel electrophoresis. This technique allows for rapid purification of the two major bands of proteins labeled by flunitrazepam. We have also prepared an affinity chromatography column with 1012-S, a new specific benzodiazepine receptor ligand received from Shionogi Res. Lab., Japan.

After purifying the BZD receptor to homogeneity, we plan to analyze its amino acid composition and prepare the monoclonal antibody. The antibody should help to establish the interaction between this BZD recognition site and GABA recognition site, and whether the benzodiazepines or beta-carbolines bind to the same or two different recognition sites.

Occupancy of benzodiazepine recognition sites by specific ligands modifies the level of anxiety, changes neuronal excitability and muscle tone, and induces sleep. A better understanding of the molecular characteristics of these sites may be of great value in determining the role of recognition sites of benzodiazepines in regulating anxiety and in understanding how these sites modify GABAergic transmission.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 01521-09 SMRP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Functional Role of Substance P and Other Peptides in Nervous System

PRINCIPAL INVESTIGATOR (List other, professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: J.P. Schwartz Research Chemist SMRP NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Molecular Biology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

0.5

0.3

0.2

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In studies on the function of substance P and on the effect of drugs on its distribution, the possibility that a pool of substance P precursor exists must be considered. The chick embryo dorsal root ganglion contains a molecular species of high molecular weight immunoreactivity which could function as a precursor in the formation of substance P. The content of this possible precursor is regulated by treatment of ganglia with nerve growth factor. Substance P, and its apparent precursor, have also been found in superior cervical ganglia, probably located in interneurons, as well as in many other tissues. Exposure of animals in utero to anti-NGF antiserum results in a loss of substance P from ganglia, spinal cord and skin, in agreement with a loss of DRG neurons. Adults exposed to anti-NGF show comparable losses of substance P content without a change in cell number.

Project Description:

In order to study the metabolism, as well as the development, of various peptidergic neurons, we have used animals exposed to antiserum against NGF. In animals exposed in utero or as newborns, there is a loss of substance P-containing cells from sensory ganglia, with a corresponding depletion of substance P in the spinal cord and skin. Preliminary results show the same sort of changes for somatostatin, another putative transmitter in sensory ganglia. In adult animals, in contrast, there is a depletion of the substance P and somatostatin content of these tissues with no loss of cell number. In addition, we find a loss of somatostatin as well as substance P in ileum and adrenal medulla. Although met-enkephalin-arg⁶-phe⁷ is found in DRG, spinal cord and ileum, its content does not change in anti-NGF exposed animals. Thus there is a specificity to the tissues and the peptides affected by NGF. The effect of anti-NGF in the adult animals is surprising since sensory ganglia have been thought to lose their NGF responsiveness during embryological development. Studies with the anti-NGF treated animals have shown that substance P-containing neurons in adrenal medulla and ileum are also NGF-responsive, whereas those of the submaxillary gland, the retina, and a variety of brain regions are not. Anti-NGF and NGF have similar effects on the substance P content of cultured human fetal sensory ganglia. In addition to looking at other peptides, use of these animals allows us to examine interactions between comodulators and between neurons.

We plan to continue these studies by measuring other peptides in order to determine how wide-spread the dependence on NGF is. In addition, we will use the animals to examine interactions between comodulators and between neurons. For example, although loss of substance P-terminals in the spinal cord had no significant effect on opiate binding, recent immunohistochemical results suggest that GABA binding may change. The potential role of peptides as neurotransmitters and/or neuromodulators in the nervous system has expanded our knowledge of how the brain functions but has also expanded the possible sites where defects or altered metabolism could result in mental disorders. It thus becomes imperative to learn as much as possible about this new class of neuroactive compounds.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01524-09 SMRP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Evidence for Peripheral Dopaminergic Neurons

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: M. Hadjiconstantinou Guest Researcher SMRP NIMH

Others: J. Xu Visiting Fellow SMRP NIMH
N.H. Neff Section Chief SMRP NIMH

COOPERATING UNITS (if any)

Medical Examiners Office, Washington, D.C.

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Biochemical Pharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MAN-YEARS:

0.5

PROFESSIONAL:

0.5

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We previously provided experimental evidence that many peripheral tissues contain rather high concentrations of dopamine suggesting that it may be a neurotransmitter in addition to being a precursor for norepinephrine. Our current objective is to provide evidence that the cardiovascular system of both rat and man contains dopaminergic neurons.

Project Description:

There is now substantial clinical and experimental evidence that dopamine receptors are found in the cardiovascular system. Activation of these receptors in vivo results in increased perfusion of some organs and a fall of blood pressure. Our objective is to provide evidence that the cardiovascular receptors are associated with dopaminergic neurons.

Blood vessels were dissected from rats and human vessels obtained at autopsy. Tissues were assayed for catecholamines by HPLC with electrochemical detection.

Dopamine and norepinephrine can be depleted differentially from the cardiovascular system of the rat with the neurotoxin 6-hydroxydopamine in combination with the amine uptake blocking drug desipramine or with the selective noradrenergic neurotoxin N-(2-chlorethyl)-N-ethyl-2-bromobenzylamine (DSP-4). Normally the percentage of dopamine compared with norepinephrine is about 6-9 percent. After treatment with DSP-4 there is a fall of norepinephrine but not dopamine, thus dopamine content can become about 70 percent of norepinephrine. Human vessels also appear to have separate stores of dopamine and norepinephrine suggesting the presence of separate neurons. For example, in the human renal artery, dopamine represents about 35 percent of norepinephrine in the tunica media and about 16 percent in the tunica intima. Norepinephrine content is similar in both layers of the vessel.

Schizophrenia and Parkinsons disease are associated with abnormal metabolism of dopamine. Some of the symptoms of these diseases may be related to a deficiency of peripheral dopaminergic neurons. Moreover, some of the side effect of drugs that act on the central dopaminergic system may be the consequences of actions on peripheral dopaminergic neurons or their receptors. Our studies are an attempt to answer some of these important questions.

The research will be terminated and the studies prepared for publication.

Publications:

Neff, N.H., Karoum, F., and Hadjiconstantinou, M.: Dopamine-containing small intensely fluorescent (SIF) cells and sympathetic ganglion function. Fed. Proc. 42: 3009-3011, 1983.

Relja, M., and Neff, N.H.: Is dopamine a peripheral neurotransmitter? Fed. Proc. 42: 2998-2999, 1983.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 01525-08 SMRP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Gene Expression and Protein Synthesis of Neural Tissues

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	I. Mocchetti	Guest Researcher	SMRP	NIMH
	J.R. Naranjo	Guest Researcher	SMRP	NIMH
Others:	J.P. Schwartz	Research Chemist	SMRP	NIMH
	E. Costa	Lab Chief	SMRP	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Molecular Biology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MAN-YEARS:

2.5

PROFESSIONAL:

2.5

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Rat C6 glioma cells contain a beta-adrenergic receptor, stimulation of which leads to an induction of a specific form of cyclic nucleotide phosphodiesterase (PDE). The induction of PDE is shown to require a rise in cyclic AMP, activation of cAMP-dependent protein kinase, translocation of the catalytic subunit of the kinase to the nucleus, phosphorylation of non-histone chromosomal proteins, and RNA polymerase II. Inhibition of one of these steps by drugs such as colchicine, cordycepin or alpha-amanitin prevents PDE induction. Direct measurement of mRNA levels can be made using cDNA probes. Treatment of bovine adrenal chromaffin cells with 8-Br-cyclic AMP results in an increase of both proenkephalin (PE) and tyrosine hydroxylase mRNA in these cells, which is time- and dose-dependent and not replicated by 8-Br-cyclic GMP. There is a comparable change in the content of enkephalin-like peptides.

Use of cDNA probes for PE and for proopiomelanocortin (POMC) has shown a differential distribution of the mRNAs in the CNS as well as differential regulation by such chronic drug treatments as haloperidol, reserpine, fenfluramine or 5,7-dihydroxytryptamine. Certain drugs alter peptide content by increasing biosynthesis of the mRNA whereas others act at the level of utilization.

Project Description:

Cyclic AMP can regulate specific gene transcription in many cell types. We have used several nervous system-derived cell lines to study this problem. The C6 glioma cell line contains a beta-adrenergic receptor through which cyclic AMP-dependent functions in the cell can be regulated. Among the consequences of isoproterenol activation of adenylate cyclase in the C6 glioma cells is an induction of cyclic AMP phosphodiesterase (PDE). The increase of PDE is a process which reaches a peak in 3-4 hrs and requires new protein synthesis. The cell cytoplasm contains 2 forms of PDE, which are separable on a DEAE-Sephacel column. The first form utilizes both cyclic AMP and cyclic GMP as substrates and is activated by calcium and calmodulin. The second form, which acts only on cyclic AMP, is specifically induced by isoproterenol treatment. Characterization of protein kinase activation is the first step in determining how the cyclic AMP-activated protein kinase is increasing the PDE content of the cells. Following activation of the cyclic AMP-dependent protein kinase, there is a translocation of the catalytic subunit of the kinase from the cytosol to the nucleus. Pretreatment of glioma cells with vinblastine or colchicine blocks the increase of nuclear protein kinase and the increase of PDE activity elicited by isoproterenol. These results suggest first that the translocation of activated subunits of protein kinase from cytosol to the nucleus is required for the induction of new synthesis of PDE molecules. In addition, since vinblastine and colchicine inhibit microtubule polymerization, the results suggest that microtubules are involved in the translocation process. Regulation of protein phosphorylation depends on activation of protein kinase, location of the activated enzyme, and specific substrates present in the sites where activated enzyme is located. An as yet unidentified nuclear acidic protein(s) represents a substrate for the translocated kinase. At 1-2 hrs following isoproterenol, there is increased phosphorylation of the acidic protein fraction, with no change in the degree of phosphorylation of either histones or the remainder of the nuclear proteins. Both the increased phosphorylation of acidic proteins and the PDE induction can be blocked by cordycepin, suggesting that acidic proteins regulate expression of the gene for PDE. RNA polymerase II is also required for induction of PDE. Its activity *in vivo* and *in vitro* as well as the induction of PDE can be blocked by either actinomycin D or alpha-amanitin.

In parallel with these studies, we have undertaken a series of experiments using cDNAs coding for human pheochromocytoma proenkephalin (PE) and rat tyrosine hydroxylase (TH) as probes and utilizing bovine adrenal chromaffin cells to study the coordinate regulation of PE and TH gene expression. Treatment of the cells with 8-Br-cyclic AMP results in increased expression of both PE and TH within one day. The effect is dose-dependent but not reproduced by 8-Br-cyclic GMP. Changes in the mRNA content are followed in time by changes in the cellular content of enkephalin-like peptides as well as high MW forms of enkephalin, increased release to the medium of the peptides, and increased TH activity. Dexamethasone also causes a dose-dependent increase of both PE and TH mRNA. In contrast, reserpine decreases catecholamines and PE mRNA, while the enkephalin peptide content increases. Previous work in the laboratory has shown a role for cyclic AMP-dependent protein kinase translocation to the nucleus in the induction of tyrosine hydroxylase in the cells. We now plan to look at the role of the protein kinase and of nuclear protein phosphorylation in the regulation of PE and TH mRNA content of the chromaffin cells. In addition we will study the effects of other opiates and neurotransmitters. This model system allows us to ask questions about the

regulation of expression of the cotransmitters, catecholamines and enkephalin peptides.

We have used cDNA probes for PE and proopiomelanocortin (POMC) to study the CNS distribution of these neuropeptide precursors and to examine the effect of various pharmacological treatments on their biosynthesis. PE mRNA can be measured in seven different brain regions, and the neurointermediate lobe of the pituitary while POMC mRNA is found in anterior and intermediate lobes of the pituitary as well as in the hypothalamus, midbrain, brainstem, cortex and cerebellum. Chronic treatment of rats with haloperidol for 2-3 weeks causes a specific two to three-fold increase in proenkephalin mRNA in striatum, with no change in other regions, which correlates with a two-fold change in the content of enkephalin-like peptides. Similar changes are seen one week following reserpine administration, or after intranigral injection of 6-hydroxydopamine. POMC mRNA increases only in the neurointermediate lobe of the pituitary after haloperidol treatment. In contrast, several drugs which affect serotonin content, fenfluramine, 5,7-dihydroxytryptamine or para-chlorophenylalanine, all increase enkephalin-like and beta-endorphin-like immunoreactive peptides in the hypothalamus and the striatum, without affecting the content of either PE or POMC mRNA. Preliminary results in rats made tolerant to morphine, or in which withdrawal is precipitated with a single injection of naloxone, suggest that the proenkephalin system is not affected but the POMC system appears to participate in tolerance. In the hypothalamus in tolerant rats, POMC mRNA decreases and this is partially reversed by naloxone. The beta-endorphin content fails to change. Further work will be required to characterize these changes completely. Thus certain neuroactive drugs can affect gene expression and thereby alter the content of opioid peptides, whereas others alter the peptide levels independent of an effect on the rate of biosynthesis, presumably through a change in peptide utilization. Furthermore, there is a great deal of specificity in terms of the specific brain regions as well as the specific mRNAs affected by any given drug.

Drugs affect neuropeptide dynamics as a result of repeated administration of daily doses. By combining the results of the assays for mRNA coding for specific neuropeptide precursors, high molecular weight precursor, and small molecular weight biologically active neuropeptides, we hope to be able to distinguish between actions of drugs on transcription, processing and release of neuropeptides.

Publications:

Tang, F., Costa, E., and Schwartz, J.P.: Increase of proenkephalin mRNA and enkephalin content of rat striatum after daily injection of haloperidol for 2 to 3 weeks. Proc. Natl. Acad. Sci. USA 80: 3841-3844, 1983.

Schwartz, J.P.: Cyclic AMP-mediated modulation of gene expression. In Hanin, I. (Ed.): Dynamics of Neurotransmitter Function. New York, Raven Press, 1984, pp. 253-263.

Schwartz, J.P., and Onali, P.: Beta-adrenergic receptor regulation of a cyclic AMP phosphodiesterase in C6 glioma cells. Adv. Cyclic Nucl. Res. 16: 195-203, 1984.

Schwartz, J.P., Quach, T.T., Tang, F., Kageyama, H., Guidotti, A., and Costa, E.: Cyclic AMP-mediated modulation of gene expression in adrenal chromaffin cell cultures. Adv. Cyclic Nucl. Res. 17: 529-534, 1984.

Quach, T.T., Tang, F., Kageyama, H., Mocchetti, I., Guidotti, A., Meek, J.L., Costa, E., and Schwartz, J.P.: Enkephalin biosynthesis in adrenal medulla: Modulation of proenkephalin mRNA content of cultured chromaffin cells by 8-Br-cyclic AMP. Mol. Pharmacol., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01526-08 SMRP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Retina: A Model for Studying Synaptic Biochemistry

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	M. Hadjiconstantinou	Guest Researcher	SMRP	NIMH
Others:	N.H. Neff	Section Chief	SMRP	NIMH
	P. Panula	Visiting Fellow	SMRP	NIMH
	A. Mariani	Staff Fellow	LVR	NEI

COOPERATING UNITS (if any)

Laboratory of Vision Research, National Eye Institute, Bethesda, MD

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Biochemical Pharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MAN-YEARS:

0.6

PROFESSIONAL:

0.6

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Our present interest is to investigate some of the properties of the epinephrine-containing neuronal system of retina and how these neurons interact with dopamine-containing neurons.

Project Description:

Dopamine, norepinephrine and epinephrine are present in mammalian retina (Z01 MH 01526-06 SMRP). Dopamine is the major catecholamine while norepinephrine and epinephrine represent about 5 percent of the dopamine content. Dopamine is contained in a subpopulation of amacrine cells and has been the subject of numerous studies by our laboratory in the past. We have also shown that norepinephrine of retina is present in the sympathetic nerves that originate from the superior cervical ganglion and innervate the retinal vessels. Our objective has been to determine the location and function of retinal epinephrine.

Immunohistochemical and HPLC methods were used to localize and determine the possible role of epinephrine in retinal function.

The enzyme for the synthesis of epinephrine, phenylethanolamine-N-methyltransferase, has been localized by an indirect immunofluorescent staining method, to a subpopulation of amacrine cells in the rat retina. The immunoreactive cells are located primarily in the inner nuclear layer and send a single process to the inner plexiform layer. Most of the immunoreactivity is found in the center of the inner plexiform layer. A small percentage of immunoreactive cell bodies were found in the inner plexiform layer and occasionally cells were observed in the ganglion cell layer. These epinephrine-containing amacrine cells are morphologically distinct from the dopamine-containing amacrine cells previously described by formaldehyde fluorescence. As the process of the epinephrine and dopamine amacrine cells were found to share common striata in the inner plexiform layer we speculated that these two neuronal systems interact.

Environmental light induces the activation of dopamine-containing neurons of rat retina and as a consequence dopamine turnover increases. The state of dopamine metabolism is directly related to the content of 3,4-dihydroxyphenylacetic acid (DOPAC) in retina. Alpha-2 adrenoceptors are present in the retina and their activation diminishes the retinal content of DOPAC of rats placed in the light, but not of rats placed in the dark. When alpha-2 antagonists are administered they increase retinal dopamine metabolism of rats in the light as well as of rats in the dark. The results are consistent with the notion that an endogenous agonist fully occupies the alpha-2 receptor in the dark and only partially occupies the receptors in the light. The most likely endogenous agonist for these receptors is epinephrine released from the newly identified population of epinephrine amacrine cells described above.

Many of the drugs that are used to treat human mental disorders modify catecholaminergic neuronal function. Our studies are providing the basis for understanding the pharmacology of these drugs as well as the side effects associated with them.

The research will be terminated and the studies prepared for publication.

Publications:

Hadjiconstantinou, M., Cohen, J., and Neff, N.H.: Epinephrine: A potential neurotransmitter in retina. J. Neurochem. 41: 1440-1444, 1983.

Hadjiconstantinou, M., Cohen, J., Rubenstein, J.R., and Neff, N.H.: An endogenous ligand modulates dopamine-containing neurons of retina via alpha-2 adrenoceptors. J. Pharmacol. Exp. Ther., in press.

Hadjiconstantinou, M., Mariani, A.P., Panula, P., Joh, T.H., and Neff, N.H.: Immunohistochemical evidence for epinephrine-containing retinal amacrine cells. Neuroscience, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 01531-07 SMRP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Nerve Growth Factors: Synthesis and Function

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	J.P. Schwartz	Research Chemist	SMRP	NIMH
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Others:	J. Byrd	Guest Researcher	SMRP	NIMH
	T.T. Quach	Visiting Associate	SMRP	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Molecular Biology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MAN-YEARS:

1.4

PROFESSIONAL:

1.4

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Recent evidence suggests that a family of nerve growth factors exist, each effective for a certain population of neurons. Human fibroblasts make a NGF similar to mouse submaxillary NGF in both its immunoreactive and biological properties. However, fibroblasts from patients with the genetically inherited disease familial dysautonomia contain a NGF which is immunoreactive but has very low biological activity. Mouse brain contains a factor which is NGF-like by immunoassay but has no biological activity. This factor increases in the cerebella of the pcd mutant mouse as the Purkinje cells die out and astrocytes proliferate. The PC12 pheochromocytoma cell line has been used to study the biological effects of NGF and whether these effects require NGF-receptor endocytosis. Transglutaminase is present in the cells and can be induced by butyrate treatment - the effect of this treatment on the NGF response is being studied.

Project Description:

Nerve growth factor (NGF), as isolated classically from the mouse submaxillary gland by Levi-Montalcini, is a protein required by certain populations of peripheral neurons for both survival and maintenance of function. Recent evidence suggests that many "nerve growth factors" exist, specific for different populations of neurons in either the CNS or PNS. A defect or loss of one of these factors would result in a disease of the nervous system.

In order to understand the role which nerve growth factor and related neurotrophic factors may play in disease states, we have undertaken a project to clone the gene for the factor(s) which appear(s) in brain following the partial ablation of entorhinal cortex. When this brain injury is performed and gelfoam is placed in the site previously occupied by the excised brain area, a trophic factor is produced by the surrounding tissue and it is released into the gelfoam. The biological properties of this factor are assayed in tissue extracts or in the liquid absorbed by the gelfoam by measuring neuron survival in dissociated cell cultures of 12-day chick embryo sympathetic or sensory ganglia. Results from such assays indicate that the factor increases after the lesion, reaching a maximum activity by five to ten days; little or no activity is present in control brain extracts. We are preparing mRNA from both control and lesioned brain to use in the preparation of a cDNA library of messages specific to lesioned brain. This will be accomplished by preparing ³²P-labeled DNA copies (cDNAs) of the mRNA of lesioned brain and removing by mRNA hybridization those sequences also expressed in control brain. The resulting library of mRNAs expressed only in lesioned brain will be screened for the factor(s) by *in vitro* translation assays as well as with a beta-NGF cDNA probe, to look for a related sequence.

Another model for studying the role of growth factors in disease is an inbred mouse strain with a genetically inheritable neurological disease, the *pcd* mutant, in which Purkinje cells develop normally but die out from day 20-50 after birth, to ask whether the proliferating astrocytes produce a CNS "NGF". Our earlier work using a CNS-derived clonal glial cell line showed that these cells made NGF and that the amount could be regulated by beta-adrenergic agonists. Our results with the mice demonstrate that there is a protein present in cerebellum which shows immunological cross-reactivity with NGF but which has no biological activity in the classic bioassay. The amount of this "NGF"-like protein increases in *pcd* cerebellum as astrocytes are proliferating. We will use the cDNA probe for NGF under relaxed conditions to attempt to identify this CNS "NGF"-like protein in order to understand its role in the normal development of the CNS and specifically the cerebellum.

In order to understand the biological effects of NGF better, studies have been initiated using the PC12 pheochromocytoma cell line which has NGF receptors and responds to NGF biochemically and biologically. These studies are centered on the question of whether the internalization of NGF along with its receptor is required for its biochemical effects. Because the enzyme transglutaminase (TGase) appears to be involved in many systems of receptor-mediated hormone endocytosis, we have measured this enzyme in PC12 cells and identified a series of inhibitors as well as an apparent inducer, butyrate, of the enzyme. We can thus manipulate the TGase activity up or down and ask whether this affects NGF endocytosis and ultimately responses of the cells to NGF. Understanding how NGF exerts its physiological effects will provide clues as to how both it and other "nerve growth factors"

function and ultimately will lead to an understanding of the role these "NGF"s may play in human mental disorders.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 01532-07 SMRP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Catecholamine Receptor

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: D.M. Chuang Chemist SMRP NIMH

Other: O. Dillon-Carter Chemist SMRP NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Monoclonal Antibody Group

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MAN-YEARS

1.4

PROFESSIONAL:

1.4

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In an attempt to get a better understanding of the interactions between α -adrenergic receptors (BAR), adenylate cyclase and membrane components, monoclonal antibodies have been raised using BAR solubilized from frog erythrocyte plasma membrane as the antigen. The immunization was performed both in vivo and in vitro. ELISA and indirect immunoprecipitation of BAR labeled with ^{125}I -iodohydroxylbenzylpindolol were used as the screening for antibody production. One of the monoclonal antibodies has been massively produced and extensively characterized. This antibody (with a chain composition of IgG-1-K) partially precipitated BAR solubilized from membranes of frog erythrocytes, frog heart and C6 glioma cells. When the antigen was fractionated by Sephadex G-150 column chromatography, two BAR binding peaks were detected; only the binding peak with higher molecular weight could be immunoprecipitated. Immunoblotting of an isoelectrofocusing gel of the antigen reveals that the antibody was bound to a single spot with a $\text{pI} \approx 6.2$. Immunoprecipitation of total erythrocyte proteins labeled with ^{125}I indicates that this antibody interacted with a protein of approximately 43,000 daltons. Internalized BAR (which was free of adenylate cyclase and guanine nucleotide binding protein) failed to be immunoprecipitated. This antibody affected biphasically basal and isoproterenol-sensitive adenylate cyclase activity in isolated plasma membranes of frog erythrocytes. At low concentration of the immunoglobulin, the cyclase activity was activated, whereas at higher concentration the activity was inhibited by the antibody. These characteristics suggest that this antibody is against a membrane component (such as guanine nucleotide binding protein) which interacts with both BAR and adenylate cyclase. This monoclonal antibody may be a useful tool for the study of the regulation of BAR function.

Project Description:

We have used the system of frog erythrocytes as a model to study the molecular events that occur in desensitization of β -adrenergic receptor (BAR) induced by exposure of erythrocytes to isoproterenol. This BAR desensitization is characterized by a reduction in the number of BAR binding sites as well as a concomitant attenuation of isoproterenol-sensitive adenylate cyclase activity. We have provided evidence that the loss of membrane-bound BAR binding sites is due to "internalization" of surface bound BAR into some intracellular compartments. These internalized BARs are devoid of the activity of catalytic unit of adenylate cyclase and are apparently free of the guanine nucleotide binding protein. When BAR is resensitized following removal of the receptor agonist from the medium, the internalized BAR is recycled to the plasma membrane. This phenomenon has been replicated by Lefkowitz and coworkers in the system of frog erythrocytes (J. Biochem. 258: 2032, 1983) and other investigators in other BAR systems (Science 210: 441, 1980; Fed. Proc. 43: 834, 1984).

Despite this information, it remains unclear as to the molecular events leading to the internalization of BAR from the plasma membrane as well as the detailed mechanisms involved in the interaction between BAR and membrane constituents for BAR to amplify the adenylate cyclase activity. In an attempt to get a better understanding of these molecular mechanisms, monoclonal antibodies to membrane components interacting with BAR have been raised using as an antigen a crude preparation of BAR solubilized from purified frog erythrocyte plasma membrane with digitonin. The spleen cells from these immunized mice were further immunized *in vitro* for three days with the antigen prior to the fusion with a line of myeloma P3 x 63 . Ag8 . 653 (which is a nonproducer of immunoglobulin). Screening for the production of desired antibody was made using enzyme-linked immunosorbent assay as well as indirect immunoprecipitation of solubilized BAR complexed to ¹²⁵I-iodohydroxylbenzylpindolol (IHYP). Following repeated limiting dilutions, desired monoclonal antibodies were massively produced by ascitic fluid from mice injected with monoclonal hybridoma cultures and immunoglobulins were isolated by DEAE-affigel blue column chromatography.

One of the monoclonal antibodies produced termed DAF has been extensively characterized. DAF has a chain composition of Ig_g1K and can immunoprecipitate 30-50% IHYP-labeled BAR solubilized from membranes of frog erythrocytes, frog heart and C6-glioma cells. However internalized BAR (which is present in the 30,000 x g supernatant derived from frog erythrocytes exposed to isoproterenol for 2 hrs and is devoid of adenylate cyclase and guanine nucleotide binding protein) fails to be immunoprecipitated. When the antigen receptor preparation is fractionated by Sephadex G-150 column chromatography, two BAR binding peaks are resolved; only the BAR present in the peak with higher molecular weight (which represents BAR bound to other membranous constituents) can be immunoprecipitated. DAF antibody does not affect cAMP accumulation in normal erythrocytes or erythrocytes stimulated with isoproterenol. However using purified plasma membranes, this antibody affects basal as well as isoproterenol-sensitive adenylate cyclase activity biphasically. At low concentrations (0.1 to 10 μ g) this antibody stimulates the activity of adenylate cyclase by about 80%, whereas at higher concentrations (>20 μ g) the cyclase activity is progressively inhibited. Normal mouse immunoglobulin does not stimulate adenylate cyclase activity under comparable conditions. Immunoblotting of an isoelectrofocusing gel of the antigen reveals that the antibody is bound to a

single spot with a $pI \approx 6.125$. Immunoprecipitation by DAF of total erythrocyte proteins labeled with NaI^{125} (by the method of peroxidase) indicates that this antibody interacts with a protein of approximately 43,000 daltons. Taken together, these data indicate that DAF is an antibody to a membrane component which interacts with both BAR as well as adenylate cyclase. Although all the available data are consistent with the view that this membrane constituent is the α subunit of the stimulatory guanine nucleotide binding protein, further characterization of this monoclonal antibody is required to verify this point.

It is well known that receptors for various neurotransmitters undergo adaptation (such as desensitization and supersensitivity) and that this receptor adaptation is vital for the regulation of receptor function and the synaptic plasticity in mental health. The present study has proven that the frog erythrocyte system is ideal for the study of the molecular mechanisms involved in the receptor desensitization as well as the amplification of the adenylate cyclase activity. Monoclonal antibodies to membranous components interacting with BAR and adenylate cyclase are particularly useful as a tool for these investigations. This study should eventually lead to a better understanding of the molecular mechanisms of receptor regulation and may provide a new basis of the therapy for some mental illness related to receptor malfunction.

Publications:

Chuang, D.M., Barbaccia, M.L., Brunello, N., and Kinnier, W.J.: Receptor regulation: An overview. In Hanin, I. (Ed.): Dynamics of Neurotransmission. New York, Raven Press, 1984, pp. 281-292.

Chuang, D.M.: β -Adrenergic receptor internalization and processing: Role of transglutaminase and lysosomes. Mol. Cell. Biochem. 58: 79-84, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01536-06 SMRP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Characterization of Receptors for Putative Neurotransmitters

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	D. Cavalla	Visiting Fellow	SMRP	NIMH
	W. Wojcik	Guest Researcher	SMRP	NIMH
Other:	N.H. Neff	Section Chief	SMRP	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Biochemical Pharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MAN-YEARS:

1.3

PROFESSIONAL:

1.3

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is to identify and study the receptors of putative transmitter substances. Our present objective is to: 1) develop photoaffinity binding ligands for receptors and 2) determine if the membrane potential of neuronal cells might be monitored with a fluorescent dye.

Project Description:

Abnormal receptor function may play a role in the etiology of some forms of mental and neurological disorders. An understanding of receptor function may give insight into such disorders. Our immediate objectives are: I) to develop photoaffinity ligands for the GABA and adenosine receptors and: II) to determine if membrane potentials of neuronal cells might be monitored with a fluorescent dye.

- I. Muscimol is a potent agonist ligand at the GABA-A receptor. Analysis of its chemical structure suggested it to be a candidate for photoaffinity labeling. Irradiation at 254 nM changed the UV spectrum of muscimol and induced an irreversible binding of tritium-muscimol to rat cerebellar synaptosomal membranes. Nonspecific binding was defined as that arising in the presence of 1 mM GABA. Specific binding increased asymptotically up to 100 nM muscimol. Dose-dependent inhibition of binding was observed with muscimol, GABA and bicuculline methiodide. Baclofen, L-glutamate and diazepam exerted no effect at high concentrations. Polyacrylamide gel electrophoresis of the photo-labeled membranes indicated specific incorporation of radioactivity into two molecular weight species. One failed to enter the separating gel, implying a molecular weight >250,000 daltons. The molecular weight of the other was about 52,000 daltons.

Tritium-2-azidoadenosine was synthesized as a potential photoaffinity label for the adenosine receptors, either A1 or A2 subtype. UV irradiation induced an irreversible attachment to plasma membrane from brain but the photo labeling was not inhibited by the adenosine receptor agonists, but was inhibited by adenosine transport blocking drugs. The 2-azidoadenosine may prove to be a useful compound for characterizing the adenosine transport protein.

- II. The fluorescent cyanine dye DiO-C5-(3) accumulates in undifferentiated neuroblastoma x glioma NG-108-15 cells to a degree dependent on their membrane potential. With a dye concentration of 25 nM, the fluorescence level increased with hyperpolarizing agents and decreased with depolarizing agents, the new equilibrium levels being established within 10 min. For example, valinomycin (1 μ M), a potassium ionophore increased fluorescence by 24 percent, potassium in a concentration dependent manner depolarized these cells. These substances had no effect on dye fluorescence if the cells were lysed by sonication.

Abnormal receptor function is assumed to be responsible for some brain disorders. Only by understanding normal receptor function and how receptor activation translates into neuronal activity can we hope to understand some neuronal diseases or how to design new therapeutic agents. Our goal is to provide the basic information needed to understand normal neuronal events.

The research will be terminated and the work prepared for publication.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 01537-06 SMRP
PERIOD COVERED October 1, 1983 to September 30, 1984		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Biochemical Pharmacology of GABA Receptor System		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	B. Wise Staff Fellow	SMRP NIMH
Others:	A. Guidotti Section Chief Y. Kataoka Visiting Fellow E. Costa Lab Chief	SMRP NIMH SMRP NIMH SMRP NIMH
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Preclinical Pharmacology		
SECTION Molecular Neurobiology		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032		
TOTAL MAN-YEARS: 0.9	PROFESSIONAL: 0.9	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>GABA-modulin is an endogenous synaptic membrane protein of 17,000 MW which inhibits the binding of 3H-GABA and the GABA-induced stimulation of 3H-diazepam binding to brain synaptic membranes. GABA-modulin is a substrate for cAMP-dependent and Ca2+-dependent protein kinases. Different sites in GABA-modulin are phosphorylated by the two classes of protein kinases. Extensive phosphorylation of GABA-modulin by the cAMP-dependent protein kinase results in the loss of its inhibitory effects on GABA binding, whereas Ca2+/calmodulin-dependent phosphorylation has no effect on its biological activity. A GABA-modulin-like protein is present in membranes prepared from bovine adrenal medulla and adrenal chromaffin cells maintained in primary culture as revealed by radioimmunoassay. Endogenous protein phosphorylation stimulated by Ca2+, Ca2+ plus phospholipid, and cAMP in adrenal medullary membranes reveals the presence of a phosphoprotein of similar molecular weight as GABA-modulin. Similarly, a phosphorylated GABA-modulin-like protein is immunoprecipitated from acid extracts of 32P-labeled chromaffin cells. Therefore, the bovine adrenal chromaffin cell may be a suitable model in which to study the regulation of GABA-modulin phosphorylation and its functional role in the GABA/benzodiazepine receptor complex.</p>		
1101		

Project Description:

When GABA receptor sites are occupied by the endogenous agonist, the ion channel located in the membrane becomes permeable to Cl^- ion, resulting in a hyperpolarization or depolarization of the receptive neuron depending on the concentration of Cl^- in the surrounding medium and intracellularly. The goal of this project is to provide a better understanding of the function and regulation of the GABA receptor complex and in particular the coupling of the GABA recognition site with the Cl^- channel as a first step in developing new potent and specific drugs which like benzodiazepines can facilitate the stimulation of GABA receptors.

Previous studies indicate that crude synaptic membranes freshly prepared from brain, purified synaptic plasma membranes, or membranes from neuroblastoma clonal cell lines contain a protein inhibitor of ^3H -GABA binding which was termed GABA-modulin (GM). Repeated washings of these membranes combined with freezing, thawing and treatment with Triton X-100 removes GM and unmasks an additional population of GABA recognition sites characterized by high affinity for the agonist. Subjecting the Scatchard plot of these binding studies to the graphic analysis of Rosenthal for one ligand and two types of binding sites, the total density of GABA receptor sites and the relative portion of high (K_d 20-40 nM) and low (K_d 200-400 nM) affinity components of ^3H -GABA binding can be estimated. GM affects the high affinity component of ^3H -GABA binding by decreasing the maximal number of such sites.

GM was purified from rat brain and characterized. It was found that GM is an excellent substrate for various protein kinases. The cAMP-dependent protein kinase incorporated up to 4 mol of phosphate per mol of GM protein, while a Ca^{2+} /calmodulin-dependent enzyme incorporated only 1 mol of phosphate per mol of protein. Different sites in GM were phosphorylated by these two enzymes. A Ca^{2+} /phospholipid-dependent enzyme phosphorylated GM at four distinct sites as revealed by HPLC of tryptic digests. Phosphorylation of GM by the cAMP-dependent mechanism resulted in a loss of GM inhibitory activity towards GABA binding, whereas the Ca^{2+} /calmodulin-dependent phosphorylation had no effect on GM activity. The effect of the Ca^{2+} /phospholipid-dependent phosphorylation on GM activity has yet to be tested.

GM present in purified synaptic membranes was phosphorylated by cAMP-, Ca^{2+} /calmodulin- and Ca^{2+} /phospholipid-dependent mechanisms. Preliminary results indicate that incubation of synaptic membranes under cAMP-dependent phosphorylation conditions results in a 30% increase in ^3H -GABA binding. Inclusion of Ca^{2+} and calmodulin, as well as cAMP, results in a greater increase in ^3H -GABA binding, whereas Ca^{2+} and calmodulin had no effect on binding. These preliminary results suggest a synergistic effect of Ca^{2+} /calmodulin- and cAMP-dependent phosphorylation on GM activity.

In order to study the regulation of GM phosphorylation by various drugs, adrenal chromaffin cells maintained in primary culture were chosen as a model system, since work by Y. Kataoka in our laboratory revealed the presence of an intrinsic GABAergic system in these cultures. In addition to the presence of GABA and diazepam binding sites in the adrenal medulla as shown by Y. Kataoka, there are also high affinity binding sites for ^{35}S -t-butylbicyclophosphorothionate, a ligand for the picrotoxin/barbiturate recognition sites of the GABA receptor complex.

GM-like immunoreactivity was found in membranes of the bovine adrenal medulla and membranes of chromaffin cells. This protein has been partially purified from adrenal medullary membranes and shows many properties similar to brain synaptic GM, such as molecular weight, HPLC profile, cation-exchange chromatography characteristics and immunoreactivity.

Labeling of chromaffin cells with ^{32}P (to label cellular ATP pools) followed by acid extraction and immunoprecipitation revealed that the GM-like protein is present in a phosphorylated state. Using SDS-polyacrylamide gel electrophoresis and autoradiography, an effect on the phosphorylation of this GM-like protein by various drugs that act on the GABA receptor could not be demonstrated in preliminary studies. A more quantitative approach to be used in the future is to separate phosphorylated proteins by HPLC followed by immunoprecipitation and radioimmunoassay. In this way, a change in the specific activity of GM phosphorylation may be detected. In fact, preliminary results using this approach indicates that incubation of cells with dibutyryl-cAMP stimulates the phosphorylation of the GM-like protein.

Endogenous protein phosphorylation in membranes of the adrenal medulla reveals a protein of similar molecular weight as GM, the phosphorylation of which was stimulated by the addition of Ca^{2+} , Ca^{2+} plus phospholipid, or cAMP. Furthermore, phosphorylation in the cytosol fraction indicates a prominent protein kinase system activated by Ca^{2+} plus phospholipid. Measurement of enzyme levels in various subcellular fractions of the adrenal medulla demonstrates the existence of the Ca^{2+} /phospholipid-dependent enzyme in the cytosol, nuclear and plasma membrane fractions, the levels of which were comparable to the ubiquitous cAMP-dependent protein kinase.

These results, coupled with those of Y. Kataoka, indicates that primary cultures of adrenal chromaffin cells are a model system in which to study GABAergic mechanisms and the regulation through a phosphorylation mechanism of the supra-molecular GABA receptor complex. Furthermore, the role of protein phosphorylation activated by cAMP or Ca^{2+} and phospholipid in the control of cellular and receptor function can be investigated in the chromaffin cell.

Publication:

Corda, M.G., and Guidotti, A.: Modulation of GABA receptor binding by Ca^{2+} . J. Neurochem. 41: 277-280, 1983.

Costa, E., Corda, M.G., Epstein, B., Forchetti, C., and Guidotti, A.: GABA-benzodiazepine interactions. In Costa, E. (Ed.): The Benzodiazepines: From Molecular Biology to Clinical Practice. New York, Raven Press, 1983, pp. 117-136.

Wise, B.C., Guidotti, A., and Costa, E.: Regulation of the GABA receptor complex by a phosphorylation mechanism. Adv. Cycl. Nucl. Protein Phosphorylation Res. 17: 511-519, 1984.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 01549-05 SMRP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Imipramine Binding Sites in Rat Brain

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: M.L. Barbaccia Guest Researcher SMRP NIMH

Other: E. Costa Lab Chief SMRP NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Molecular Neurobiology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MAN-YEARS:

0.6

PROFESSIONAL:

0.6

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A selective lesion of the 5-HT axon terminals carried out by injecting i.c.v. 5,7-dihydroxytryptamine (5,7-DHT), prevents the down-regulation of β -adrenergic receptors measured in cortical minces after repeated daily injections of imipramine or desipramine. This suggests a functional link (neuronal loop?) connecting 5-HT axons with NE synapses which is operative in the regulation of the NE-receptor function and perhaps participates in the antidepressant action of imipramine and its congeners. The V_{max} of the 5-HT reuptake by hippocampal minces is increased when the number of imipramine binding sites is decreased by daily injections of imipramine or desipramine repeated for two to three weeks. Moreover in these minces also the in vitro inhibition of the 5-HT uptake by various imipramine concentrations is attenuated. These findings are consistent with a physiological role of the imipramine binding site and support the working hypothesis that an endogenous effector (endocoid) modulates 5-HT uptake by acting on imipramine recognition sites. A thermally stable, nonpeptidic, endocoid that selectively inhibits in a dose-dependent manner 3H-5-HT uptake and 3H-imipramine binding was purified from brain. Crude synaptic membranes of rat brain contain also specific and high affinity binding sites for 3H-mianserin, an atypical antidepressant. The 3H-mianserin recognition sites appear to be different from the 5-HT₂ recognition sites labeled by 3H-ketanserin. A 5,7-DHT lesion increases the number of 3H-mianserin recognition sites, while leaves unchanged the 3H-ketanserin sites. The mianserin recognition site appears to be a modulatory site distinct from the 5-HT₂ recognition site but cooperating to modulate serotonergic synapses.

Project Description:

Though antidepressant drugs have been used extensively in the treatment of depression, neither the molecular mechanisms of the drug action nor the etiology of the disease is well understood. Recently, high affinity binding sites specific for typical antidepressants such as imipramine and desipramine and atypical antidepressants such as mianserin have been reported to be present in various brain structures of several species. These discoveries have provided a new tool to study the molecular events involved in the therapeutic effects of antidepressant drugs. Various investigators have suggested that desensitization of β -adrenergic receptors (or decrease in NE-sensitive adenylate cyclase) in brain of rats after chronic treatment with antidepressants is most likely linked to their therapeutic action. Selective lesion of 5-HT axon terminals were carried out by injecting i.c.v. 5,7-dihydroxytryptamine (5,7-DHT). To evaluate the role of imipramine binding sites, which have been shown by us and others to be present on 5-HT axon terminals, on the down-regulation of NE-sensitive adenylate cyclase induced by protracted imipramine or desipramine treatment, we have found that this 5,7-DHT lesion prevents the loss of β -adrenergic recognition sites as well as the attenuation of the responsiveness of adenylate cyclase to isoproterenol in isolated cortical membranes of rats chronically treated with desipramine. Moreover the attenuation of the NE-sensitive cAMP generating system in cortical minces induced by imipramine is also prevented by lesion of 5-HT nerve terminals. In contrast, down-regulation of the NE-stimulated cAMP accumulation in cortical slices elicited by repeated administrations with mianserin is unaffected by 5,7-DHT lesion. These results suggest that a neuronal regulatory loop might connect 5-HT terminals with the neurons where β -adrenergic receptors are located and that this link participates in the attenuation of NE-receptor function and, perhaps, in the antidepressant action of imipramine and related drugs.

Several lines of evidence indicate that imipramine binding sites are related to a regulatory site of 5-HT uptake system. We have found that the B_{max} of 3H -imipramine binding to crude synaptic membranes prepared from hippocampi of rats receiving imipramine (twice daily for 1 to 3 weeks) is reduced whereas the net uptake of 5-HT (V_{max}) by hippocampal minces is increased. Also the inhibitory effect on the 5-HT uptake by various imipramine concentrations added "in vitro" to the hippocampal minces is attenuated when the number of 3H -imipramine binding sites is decreased by repeated imipramine injections. Our data support the possibility that the sites where 3H -imipramine binds play a physiological role for the regulation of 5-HT uptake. Hence 5-HT uptake system functions as a supramolecular entity where various subunits are involved in the fine tuning of the uptake process. In support of these inferences we have partially purified a thermostable nonpeptidic endogenous effector of 3H -imipramine binding sites from rat brain which, in a dose dependent manner, inhibits 5-HT uptake and displaces 3H -imipramine binding. We have tested this putative endocoid on 3H -flunitrazepam, 3H -mianserin, 3H -dihydroalprenolol binding and none of these ligands could be displaced by the inhibitor of 3H -imipramine binding. The endocoid extracted from rat brain appears to have a molecular weight of $<1,800$ daltons, it is polar (basic), is soluble in methanol and ethanol, not in propanol or acetonitrile, it can be separated from 5-HT by HPLC on a reverse phase column. On the basis of their 1) HPLC retention time, 2) GC/MS fragmentographic pattern, 3) IC_{50} values in displacing 3H -imipramine bound to crude synaptic membranes, the following indolealkyl derivatives can be ruled out as possible candidates for the role of the putative endocoid for the imipramine

recognition site: 5-HIAA, 5-hydroxytryptophol, tryptophol, kynuramine, D-L kynurenine, kynurenic acid, methyl- β -carboline, harmol, harmaline, harmine, norharmine, harmene, harmalol, tryptamine, 5-methoxytryptamine, N-acetyl serotonin. Among the compounds tested only the 6-hydroxy- and 6-methoxy-tetrahydro- β -carboline showed a good inhibitory activity on ^3H -imipramine binding and 5-HT uptake (IC_{50} s 0.8 and $0.2 \mu\text{M}$ and 0.6 and $0.8 \mu\text{M}$, respectively). However GC/MS and HPLC elution characteristics of these compounds seem to rule out the possibility of their identity to the putative endocoid which is being extracted from rat brain. Preliminary results obtained in GC/MS seem to favor the possibility that a sort of tricyclic structure with the following elemental composition: $\text{C}_{14}\text{H}_{17}\text{N}_3\text{O}_4$ might be present in the rat brain extract that contains the putative endocoid for ^3H -imipramine binding. We are now in the process of synthesizing this compound in order to test its biological activity. Once identified this effector may be of importance as a biochemical marker to study the action of imipramine and to study the molecular nature of the biochemical defect(s) operative in certain types of depression.

Crude synaptic membranes of rat brain contain specific high affinity binding sites for an atypical antidepressant, mianserin. It was previously suggested that ^3H -mianserin labels 5-HT₂ receptor recognition sites because this binding can be effectively displaced by spiroperidol and ketanserin (*J. Pharmacol. Exp. Ther.* 216:142-148, 1981). However we have shown that the number of ^3H -mianserin binding sites are increased following lesion of 5-HT axons with 5,7-dihydroxytryptamine whereas the binding characteristics of ^3H -ketanserin remain unchanged. Moreover repeated daily injections of imipramine decrease the specific binding of ^3H -ketanserin but fail to affect the binding of ^3H -mianserin to crude synaptic membranes prepared from rat hippocampus or cortex. The ^3H -mianserin and ^3H -ketanserin recognition sites adapt differently to a chronic treatment with ketanserin or mianserin itself. One single dose of mianserin given 48 hrs before killing the rats down-regulates the B_{max} of ^3H -ketanserin binding, while does not affect the ^3H -mianserin binding kinetic characteristics. Mianserin can decrease the B_{max} of its own recognition sites only after 3 weeks of repeated daily injections. On the contrary one single administration of ketanserin 48 hrs before killing the rats does not affect either ^3H -mianserin or ^3H -ketanserin binding. Only after 7 days of repeated daily injections ketanserin down-regulates ^3H -ketanserin recognition sites while it does not change the ^3H -mianserin binding, not even after 21 days of repeated daily treatments. Both mianserin and ketanserin given for 3 weeks, elicit an attenuation of the NE-stimulated cAMP accumulation in cortical slices. These results suggest that the binding sites for mianserin and 5-HT₂ recognition sites are not identical but they may interact allosterically. A working hypothesis is that 5-HT axons produce 2 chemical signals, each one of them acting on a different synapse. One is serotonergic, the other has 2 specific recognition sites, one for the signal produced by the 5-HT axon that acts through the ^3H -mianserin binding site; the other labeled by ^3H -ketanserin or ^3H -spiroperidol which is called 5-HT₂ receptor and is modulated by the effector produced by 5-HT axons that binds on ^3H -mianserin binding site. We are currently trying to verify this model by studying the interactions between the ^3H -ketanserin and the ^3H -mianserin binding sites and by trying to isolate the possible endogenous effector for the ^3H -mianserin site.

We have also studied the mechanisms of action of two other atypical antidepressants, iprindole and bupropion. Repeated daily injections of iprindole for 21 days decrease the density of α -adrenergic receptor binding sites and NE-sensitive adenylate cyclase activity in the frontal cortex. However these iprindole-induced

events are unaffected by a lesion of 5-HT axon terminals. Repeated but not acute administrations of iprindole decrease the number of ^3H -ketanserin and ^3H -mianserin binding sites in the frontal cortex and hippocampus but do not modify the binding characteristics of ^3H -5-HT₁ receptor recognition sites. The time course of the modifications of ^3H -mianserin and ^3H -ketanserin binding after iprindole show that while the Bmax of ^3H -ketanserin binding is decreased already after 4 days of treatment, the Bmax of ^3H -mianserin binding is decreased only after 1 week of treatment. Moreover while the decrease of ^3H -ketanserin binding elicited by repeated imipramine injections is prevented by a selective lesion of the 5-HT axon terminals, the decrease of ^3H -ketanserin and ^3H -mianserin binding evoked by iprindole are not sensitive to serotonergic denervation. Since "in vitro" experiments have shown that iprindole can displace the ^3H -mianserin bound to its recognition sites with an IC₅₀ value which is lower than that necessary to displace other ligands one could surmise that iprindole may be acting directly on the postsynaptic ^3H -mianserin and/or ^3H -ketanserin recognition sites. Bupropion was considered to be an atypical antidepressant because it was reported by others that this drug upon chronic treatments fails to modify the function of β -adrenergic receptors. However we found that in the brain of rats treated with relatively high doses of bupropion (50 mg/kg, twice daily) for 3 weeks, the density of β -adrenergic receptor recognition sites and the activity of NE-sensitive adenylate cyclase are both attenuated. The specificity of this bupropion effect is supported by the finding that the binding of ^3H -mianserin, ^3H -ketanserin and ^3H -5-HT to crude synaptic membranes is unaffected following long-term bupropion administration. Currently we are studying the molecular mechanisms of the bupropion elicited down-regulation of β -adrenergic receptor function.

The present study has moved an important step toward the understanding of the etiology of mental depression and the therapeutic action of several antidepressant drugs. The endogenous ligands for imipramine and mianserin binding sites in CNS may be causally related to the disease state of certain forms of mental depression and their levels in the cerebral spinal fluid may therefore be used as a biochemical marker of depression. Further purification and characterization of these endogenous ligands and the search for other classes of endogenous ligands are now in progress. These studies could lead to formulation for a better therapy of affective disorders.

Publications:

Barbaccia, M.L., Chuang, D.M., Gandolfi, O., and Costa, E.: Transsynaptic mechanisms in the action of imipramine. In Usdin, E., and Stephanis, C. (Eds.): Frontiers in Neuropsychiatric Research. Houndmill, U.K., Macmillan Press, 1983, pp. 19-31.

Costa, E., Chuang, D.M., Barbaccia, M.L., and Gandolfi, O.: Molecular mechanisms in the action of imipramine. Experientia 39: 855-858, 1983.

Barbaccia, M.L., Gandolfi, O., Chuang, D.M., and Costa, E.: Modulation of neuronal 5-HT uptake by a putative endogenous ligand of imipramine recognition sites. Proc. Natl. Acad. Sci. USA 80: 5134-5138, 1983.

Gandolfi, O., Barbaccia, M.L., Chuang, D.M., and Costa, E.: Daily bupropion injections for 3 weeks attenuate the NE-stimulation of adenylate cyclase and the number

of β -adrenergic recognition sites in rat frontal cortex. Neuropharmacology 22: 927-929, 1983.

Costa, E., Barbaccia, M.L., Gandolfi, O., and Chuang, D.M.: Endogenous modulation of serotonin uptake as a site for the action of imipramine. In Biggio, G., and Costa, E. (Eds.): Advances in Biochemical Psychopharmacology. New York, Raven Press, in press.

Barbaccia, M.L., Karoum, F., Gandolfi, O., Chuang, D.-M., and Costa, E.: Putative endogenous ligands for antidepressant recognition sites. Proceedings of the 14th C.I.N.P. Meeting, in press.

Barbaccia, M.L., and Costa, E.: Autacoids for drug receptors: A new approach in drug development. New York Acad. Sci., in press.

Gandolfi, O., Barbaccia, M.L., and Costa, E.: Comparison of iprindole, imipramine and mianserin action on brain serotonergic and β -adrenergic receptors. J. Pharmacol. Exp. Ther., in press.

Gandolfi, O., Barbaccia, M.L., and Costa, E.: Differences between ^3H -mianserin and ^3H -ketanserin recognition sites. Life Sci., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
 NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01550-04 SMRP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biochemical Mechanisms Regulated by Various Receptors in Anterior Pituitary

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	P. Onali	Visiting Associate	SMRP	NIMH
Others:	M. Olianias	Visiting Associate	SMRP	NIMH
	C. Eva	Visiting Fellow	SMRP	NIMH
	J.P. Schwartz	Research Chemist	SMRP	NIMH
	E. Costa	Lab Chief	SMRP	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Molecular Biology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MAN-YEARS:

2.3

PROFESSIONAL:

1.5

OTHER:

0.8

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The interaction of stimulatory and inhibitory receptors at the level of adenylate cyclase has been studied in three systems. In rat anterior pituitary, vasoactive intestinal peptide (VIP) stimulates adenylate cyclase and prolactin release in the mammotrophs. Dopamine can block both of these responses through action at a D-2 receptor. Cholera toxin also activates adenylate cyclase and DA can block this effect by acting on a D-2 receptor. The rat pituitary GH3 cell line provides a single population of cells which also respond to VIP with both adenylate cyclase activation and prolactin secretion. Muscarinic agonists can inhibit both basal and VIP-stimulated adenylate cyclase, as well as prolactin secretion, in this cell line. Changes in cyclase activity correlate with changes in cyclic AMP content and in prolactin release, suggesting that cyclic AMP serves as one second messenger for regulation of PRL secretion. Muscarinic receptors in rat striatum also inhibit adenylate cyclase and concurrently stimulate a high affinity GTPase, suggesting that inhibitory receptors may affect adenylate cyclase via action on a GTPase. The GH3 cells will allow us to examine this question further in a pure cell population.

Proposed Course:

This project has been terminated.

Publications:

Onali, P., Schwartz, J.P., and Costa, E.: Inhibitory coupling of dopamine receptors to adenylate cyclase in rat anterior pituitary. In Biggio, G., Costa, E., Gessa, G., and Spano, P.F. (Eds.): Receptors as Supramolecular Entities. England, Pergamon Press, 1983, pp. 51-59.

Onali, P., Eva, C., Olanas, M.C., Schwartz, J.P., and Costa, E.: In GH3 pituitary cells acetylcholine and vasoactive intestinal peptide (VIP) antagonistically modulate adenylate cyclase, cyclic AMP content and prolactin secretion. Mol. Pharmacol. 24: 189-194, 1983.

Onali, P., Olanas, M.C., Schwartz, J.P., and Costa, E.: Involvement of a high affinity GTPase in the inhibitory coupling of striatal muscarinic receptors to adenylate cyclase. Mol. Pharmacol. 24: 380-386, 1983.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 01552-04 SMRP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Agonist and Antagonist of Benzodiazepine Receptors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	M. Ferrari	Guest Researcher	SMRP	NIMH
Others:	M.G. Corda	Guest Researcher	SMRP	NIMH
	A. Guidotti	Section Chief	SMRP	NIMH
	E. Costa	Lab Chief	SMRP	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

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TOTAL MAN-YEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Binding of benzodiazepine recognition sites by various ligands can elicit opposite types of responses, such as proconvulsant/anticonvulsant, or anxiogenic/anxiolytic. In order to answer whether a new class of drug belongs to benzodiazepine (anxiolytic), or beta-carboline (anxiogenic) type of ligand we developed a behavioral animal model that predicts the anxiogenic and anxiolytic potency of a drug. The inhibition of the response by the pure antagonist (partial agonist?) of the benzodiazepine recognition site assumes that proconflict and anticonflict action depends on the binding of the drug to the benzodiazepine recognition site.

Project Description:

In order to differentiate between proconflict and anticonflict action of drugs, we adapted the behavioral paradigm developed by Vogel to screen anticonflict action of drugs. Water-deprived rats were placed in the experimental chamber where they had access to water through a stainless steel drinking tube extending 2 cm into the chamber. In absence of aversive stimulus, animals licked without interruption for the testing period (3 min) totaling approximately 28-30 drinking periods (one drinking period is equal to 3 sec of cumulative drinkometer output). A conflict situation which suppresses responding was induced by applying an electric shock at the end of each 3 sec drinking period. A current intensity of 1 mA reduced the number of drinking periods by 80% or more. Diazepam and other anxiolytic benzodiazepines reduced in a dose-dependent manner this behavioral inhibition induced by punishment.

To evaluate the proconflict action of a drug and to rank the potency of such drugs, the intensity of the aversive stimulus (electric shock) applied to the water spout was reduced to an intensity that would leave almost unchanged spontaneous water drinking. Hence the proconflict action of a drug could be evaluated by its capability as behavioral inhibitor to increase the action of punishment. We noticed that if the intensity of the electric shock was reduced from 1 mA to 0.20-0.30 mA, the sensitization of punished behavior became suitable to evaluate the potency of drugs which produce proconflict action.

Among the drugs studied was FG 7142 (beta-carboline-3-carboxylic acid ethyl-ester methylamide) which is anxiogenic in man, as well as other beta-carboline derivatives. All the drugs elicited proconflict action, while RO 15-1788 and CGS 8216 (2-phenylpyrazolo[4,3]-quinolin-3[5H]one) antagonize the proconflict action of FG 7142 or that of other beta-carbolines. The proconflict response induced by beta-carboline derivatives was elicited by doses significantly lower than those reducing unpunished drinking and were not due to change in thirst or pain threshold or to electrical seizures of selected brain structures that could be detected only through an EEG study. These findings suggest that chemicals acting as ligands of benzodiazepine receptors can be classified into three categories:

1. Ligands with anticonflict action. These diazepam-like drugs are ineffective in the proconflict test but they block proconflict action of beta-carboline derivatives.
2. Ligands with proconflict action. These drugs, such as the beta-carboline derivative FG 7142, have no effect in the anticonflict test but block the effect of diazepam.
3. Ligands with neither proconflict nor anticonflict action. These drugs (RO 15-1788 and CGS 8216) are, however, able to block the anticonflict and proconflict actions of benzodiazepine and beta-carboline derivatives, respectively.

Isoniazid, a drug that decreases GABAergic transmission by blocking GABA synthesis, potentiates the action of beta-carboline derivatives in the proconflict test. In animals treated with isoniazid, RO 15-1788 is no longer inert but shows an intrinsic activity similar to that of beta-carboline (i.e. proconflict). This action of RO 15-1788 is observed in doses of 10-15 mg/kg i.p. Moreover,

pentylentetrazol, a drug that selectively antagonizes the GABA-mediated post synaptic inhibition by blocking Cl^- conductance (McDonald and Barker, 1978), acts as a potent proconflict agent. This suggests that the proconflict action is elicited by a down regulation of GABA receptor function.

The importance of the present observation is that these proconflict and anti-conflict tests can be used to further investigate whether drug-induced shifts in conflict behavior are correlated with functional interactions between benzodiazepine recognition sites and the various structural components of GABA receptors, and whether the activity of the drug in this test correlates with anxiogenic or anxiolytic potency in man.

This behavioral test can be a powerful tool in identifying benzodiazepine ligands with potential anxiolytic or anxiogenic activity. This test has also been used for to study the behavioral effects of endogenous ligand for benzodiazepines.

If the effect of drugs on this animal behavioral test should relate to their clinical efficacy as anxiolytic or anxiogenic agents, the test will help to study drugs inducing pathological anxiety and to screen possible therapeutic applications of agonists and antagonists of benzodiazepine receptors.

With this model test, we intend to study a series of benzodiazepine recognition site ligands endowed with agonistic and antagonistic activity and then correlate their potency in this test with their potency in facilitating and disfacilitating the 3H -muscimol binding in vivo. We also intend to use this test to study the role of GABA in anxiety and to screen for possible endocoids of the benzodiazepine recognition sites.

Publication:

Corda, M.G., Costa, E., and Guidotti, A.: Involvement of GABA in the facilitation of punishment suppressed behavior induced by beta-carbolines in rat. In Biggio, G., and Costa, E. (Eds.): Benzodiazepine Recognition Site Ligands: Biochemistry and Pharmacology. New York, Raven Press, 1983, pp. 121-128.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
 NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01553-04 SMRP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biosynthesis of Enkephalins in Bovine Adrenal Medulla

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	I. Lindberg	Staff Fellow	SMRP	NIMH
Others:	H.-Y.T. Yang	Section Chief	SMRP	NIMH
	J. Pierce	Section Chief	LCH	NHLBI

COOPERATING UNITS (if any)

Animal Medicine and Surgery Section, Lab. Chemistry, NHLBI, Bethesda, Md.

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Neuropeptide Section

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MAN-YEARS:

1.0	PROFESSIONAL: 1.0	OTHER: 0
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CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The work accomplished during this year falls into two categories. The first represents a continuation of our investigation into the enzymatic mechanisms responsible for the production of met5-enkephalin in adrenal chromaffin cells. We have further characterized a trypsin-like serine protease present in adrenal chromaffin granules by studying reactivity of this enzyme toward proenkephalin-derived peptides purified from chromaffin granules. Cleavage at pairs of basic vs. single basic amino acids was assessed using the 5.3 K dalton fragment as a substrate. The enzyme cleaved this peptide after a pair of basic amino acids, thus generating met5-enk-arg6-gly7-leu8 (MERGL); no cleavage at the single arginine in MERGL was observed. Thus the enzyme exhibits limited specificity which would be expected for a prohormone processing enzyme. Purified human HMW and LMW kininogen were also used as substrates for the enzyme; no cleavage of kininogens to bradykinin was observed, suggesting that human kininogens are not recognized by this enzyme.

In addition the release of the various molecular weight forms of the octapeptide MERGL from brain tissue was studied. Brain slices prepared from areas which were known from previous work to contain both high and low molecular weight forms of MERGL-IR peptides were found to release both of these forms following stimulation with high potassium. Gel filtration of released immunoreactivity revealed that 63% of the IR released from slices of rat medulla-pons was in the high molecular weight form. Approximately half of the immunoreactive molecules released from slices of rat hypothalamus were present as the high molecular weight MERGL-IR peptide. These results suggest further investigation directed to ascertain whether this peptide functions as a neuromodulator in addition to its role as a precursor for MERGL.

Project Description:

Previous work has involved the partial purification of a serine protease from adrenomedullary chromaffin granules. This enzyme was shown to be capable of generating met⁵-enkephalin from a pool of high molecular weight precursor(s). In the past year, I have used purified proenkephalin-derived peptides to explore the substrate cleavage site and specificity of this enzyme. The peptides were purified from an acid extract of adrenal chromaffin granules by gel filtration and repetitive HPLC. Cleavage at pairs of basic vs. single basic amino acids was examined using the 5.3 kDal peptide as a substrate. The 5.3 kDal peptide contains the sequence met⁵-enkephalin-arg⁶-gly⁷-leu⁸ at the carboxyl terminus; it was cleaved by the adrenal enzyme to form met⁵-enkephalin-arg⁶-gly⁷-leu⁸. This cleavage site implies a preference on the part of the enzyme for pairs of basic amino acids, since the single arginine present in met⁵-enkephalin-arg⁶-gly⁷-leu⁸ was not cleaved. When the products of digestion were subjected to HPLC and cleaved peptides monitored by U.V. absorption, it was found that the number of peptides generated was small compared to tryptic digests. These results strengthen the hypothesis that this enzyme is not a general protease, but possesses the limited specificity that would be expected of a prohormone processing enzyme. The possibility that this enzyme is of the kallikrein family was tested by incubating enzyme together with human high molecular weight kininogen (obtained from Dr. Jack Pierce, N.I.H.). The production of bradykinin was monitored by radioimmunoassay. No immunoreactive bradykinin was liberated from kininogen even after lengthy incubations. Thus either 1) human kininogen is not a substrate for this enzyme; or 2) the enzyme is not a kallikrein.

Other related studies performed during the past year have focused on the investigation of the release of high molecular weight forms of met⁵-enkephalin-arg⁶-gly⁷-leu⁸ (MERGL) from various regions of rat brain. It was found that slices prepared from rat medulla-pons release high molecular weight forms of MERGL-IR following depolarization with potassium (40% of total released). Slices prepared from rat hypothalamus also released substantial quantities of high molecular weight MERGL-IR in response to potassium depolarization (40-60% of total released). It is possible that this high molecular weight form of MERGL does not merely represent a precursor to MERGL, but has a modulatory role of its own.

Significance to Biomedical Research

The present investigation is clearly relevant to biomedical research in that it represents an attempt to understand the enzymatic mechanisms responsible for the biosynthesis of an important neuropeptide, met⁵-enkephalin. Once the enzymatic processes by which the enkephalins are synthesized are identified, it will become possible to study the regulation of proenkephalin processing through a study of the effects of pharmacologic manipulation of enkephalin-generating enzymes. Purification of the serine protease will be continued.

Publications:

Lindberg, I., Yang, H.-Y.T., and Costa, E.: A high molecular weight form of met⁵-enkephalin-arg⁶-gly⁷-leu⁸ in rat brain and bovine adrenal chromaffin granules. Life Sci. 33: Suppl. 1, 5-8, 1983.

Lindberg, I., Yang, H.-Y.T., and Costa, E.: Further characterization of an enkephalin-generating enzyme from bovine adrenal chromaffin granules. J. Neurochem. 42: 1411-1419, 1984.

Lindberg, I., and Yang, H.-Y.T.: Distribution of met⁵-enkephalin-arg⁶-gly⁷-leu⁸ immunoreactive peptides in rat brain: Presence of multiple immunoreactive forms. Brain Res. 299: 73-78, 1984.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 01555-04 SMRP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Enkephalin Metabolism

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	B. Mellstrom	Visiting Fellow	SMRP	NIMH
Others:	H.-Y.T. Yang	Section Chief	SMRP	NIMH
	J. Chou	Visiting Fellow	SMRP	NIMH
	J. Tang	Visiting Fellow	SMRP	NIMH

COOPERATING UNITS (if any)

Dr. S. Blumberg, Weizmann Institute of Science, Rehovot, Israel

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Neuropeptide Section

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MAN-YEARS:

1.3

PROFESSIONAL:

1.3

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Previously we have demonstrated that met-enkephalin-arg-phe (YGGFMRF) is metabolized by dipeptidyl carboxypeptidase and aminopeptidase. With the cooperation of Dr. S. Blumberg from the Weizmann Institute of Science, Rehovot, Israel, we have continued to search for effective enzyme inhibitors to block the YGGFMRF inactivation. Three mercaptoacylated dipeptides, HS-CH₂-CO-Leu-Phe, HS-CH₂-CO-Phe-Leu and HS-CH₂-CO-Leu-D-Phe were provided by Dr. Blumberg as potential enzyme inhibitors. HS-CH₂-CO-Leu-Phe effectively inhibited the inactivation of YGGFMRF by dipeptidyl carboxypeptidase in vitro. To estimate the in vivo effect of this inhibitor, the recovery of YGGFMRF released by substance P into spinal cord subarachnoidal space was measured. HS-CH₂-CO-Leu-Phe, when added into the perfusion medium, was found to increase the release of YGGFMRF. Interestingly, the recovery of YGGFMRF released into spinal cord was increased by subcutaneous injection of CH₃-CO-S-CH₂-CO-Leu-Phe-OCH₃. Whether this inhibitor is capable of reaching CNS after subcutaneous injection still remains to be further studied.

Project Description:

It was previously demonstrated that dipeptidyl carboxypeptidase and aminopeptidase participate in the metabolism of met-enkephalin-arg-phe (YGGFMRF). Substance P infused into the subarachnoidal space of the rat spinal cord releases YGGFMRF and met-enkephalin (YGGFM). The recovery of released YGGFMRF but not that of YGGFM was increased when captopril was infused into the subarachnoidal space of the spinal cord. The result further support an important role of dipeptidyl carboxypeptidase in YGGFMRF inactivation in the subarachnoidal space or in the spinal cord. Though captopril is highly effective in inhibiting YGGFMRF metabolism, this drug cannot penetrate the blood-brain barrier, readily. In this study, with the cooperation of Dr. S. Blumberg, we have continued to search for effective enzyme inhibitors to block the YGGFMRF inactivation.

In the in vitro study, a microsomal preparation from rat striatum was used as the dipeptidyl carboxypeptidase source and enzyme reaction was determined by measuring the enzymatic products using reverse phase HPLC. Three mercaptoacylated dipeptides, HS-CH₂-CO-Leu-Phe, HS-CH₂-CO-Phe-Leu and HS-CH₂-CO-Leu-D-Phe were provided by Dr. Blumberg as potential enzyme inhibitors. HS-CH₂-CO-Leu-Phe effectively inhibited the hydrolysis of YGGFMRF by dipeptidyl carboxypeptidase in vitro. To estimate the in vivo effect of this inhibitor, subarachnoidal space of rat spinal cord was perfused with artificial CSF using a push and pull cannula as described by Yaksh et al. (*J. Neurophysiol.* 46:1056-1075, 1981) and the release of YGGFMRF and YGGFM was induced by perfusing the subarachnoidal space of the spinal cord with 10⁻⁹M substance P. YGGFMRF released was measured by radioimmunoassay. When HS-CH₂-CO-Leu-Phe and bestatin were added into the perfusion medium, the recovery of YGGFMRF but not that of YGGFM was increased. In searching for inhibitors capable of penetrating the blood-brain barrier, CH₃-CO-S-CH₂-CO-Leu-Phe-OCH₃, a less polar compound, was tested. Interestingly, the recovery of YGGFMRF released into spinal cord was increased by subcutaneous injections of CH₃-CO-S-CH₂-CO-Leu-Phe-OCH₃. It is possible that CH₃-CO-S-CH₂-CO-Leu-Phe-OCH₃ may be converted enzymatically to HS-CH₂-CO-Leu-Phe in vivo and thereby inhibit the inactivation of released YGGFMRF. Whether this less polar inhibitor, CH₃-CO-S-CH₂-CO-Leu-Phe-OCH₃ is capable of reaching CNS after subcutaneous injection still remains to be further studied. We are also testing a series of potential dipeptidyl carboxypeptidase inhibitor provided by Revlon and Hoechst.

It is important to obtain inhibitors of YGGFMRF inactivation which are capable of penetrating the blood-brain barrier because these inhibitors could reveal the functional profile of YGGFMRF and possess important pharmacological actions.

The proposed course of this study is 1) to continue searching for efficient inhibitors capable of crossing the blood-brain barrier and 2) to study the catabolism of other opioid peptides such as met-enkephalin-arg-gly-leu and dynorphin(1-8).

Publications:

Zhang, A.Z., Tang, J., Chou, J., Yang, H.-Y.T., and Costa, E.: Met⁵-enkephalin-Arg⁰-Phe: Neurochemical and neuropharmacological considerations. In Ehrenpreis, S., and Sicuten, F. (Eds.): Degradation of Endogenous Opioids: Its Relevance in Human Pathology and Therapy. New York, Raven Press, 1983, pp. 1-10.

Tang, J., Chou, J., Yang, H.-Y.T., and Costa, E.: The effect of peptidase inhibitors on the release of met⁵-enk-arg⁶-phe (YGGFMRF) and met⁵-enkephalin (YGGFM) from spinal cord induced by substance P in vivo. Life Sci. 33: Suppl. I, 121-124, 1983.

Chou, J., Tang, J., Del Rio, J., Yang, H.-Y.T., and Costa, E.: Action of peptidase inhibitors on methionine-enkephalin-arginine⁶-phenylalanine (YGGFMRF) and methionine⁵-enkephalin (YGGFM) metabolism and on electroacupuncture antinociception. J. Pharmacol. Exp. Ther., in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 01558-03 SMRP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunohistochemical Studies on Neurotransmitters in the Nervous System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	P. Panula	Visiting Fellow	SMRP	NIMH
Others:	H.-Y.T. Yang	Section Chief	SMRP	NIMH
	E. Costa	Lab Chief	SMRP	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Molecular Neurobiology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 2032

TOTAL MAN-YEARS:

1.1	PROFESSIONAL: 1.1	OTHER: 0
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CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Immunohistochemical localization of bombesin or gastrin releasing peptide (BN/GRP-IR) and substance P in consecutive sections of rat hypothalamus was studied. BN/GRP-IR in the hypothalamus was characterized by gel filtration column chromatography.

The results of this comparative immunohistochemical study show that there are major differences in the distribution and number of neurons exhibiting substance P and BN/GRP-IR in the rat hypothalamus. Cells which exhibit substance P-immunoreactivity (IR) are distributed throughout the hypothalamus whereas only two nuclei (nucleus supra-chiasmaticus and nucleus paraventricularis) contain large numbers of cells with BN/GRP-IR. The number of substance P-IR cells is also considerably larger than that of BN/GRP-IR cells. The BN/GRP-IR material is not identical to BN but is chromatographically identical to GRP(1-27) and GRP(18-27).

The highest concentration of opioid peptide met-enkephalin-arg-gly-leu (YGGFMRGL) was found in the dorsal horn of the rat spinal cord. A dense network of perikarya and fibers was found in laminae I and II of the dorsal horn. Cell bodies were frequently observed in laminae IV. Additional terminals were seen around the central canal and in the ventral grey matter, often outlining perikarya of motor neurons. This localization of YGGFMRGL in spinal cords suggest a role for this peptide in sensory-motor integration.

Project Description:

This is a continuation of the work initiated to locate neuropeptides by immunohistochemical techniques in the central and peripheral nervous system to understand the interrelationships of these neuronal systems.

Immunohistochemical localization of bombesin or gastrin-releasing peptide like immunoreactivity (BN/GRP-IR) and substance P immunoreactivity (SP-IR) in rat hypothalamus was studied. In rat hypothalamus, SP was more widely distributed than BN/GRP-IR. Only the anterior and medial parvocellular parts of the nucleus paraventricularis and the nucleus supraopticus contained numerous cell bodies containing BN/GRP-IR. SP positive cell bodies were not found in these areas. In contrast, SP positive cell bodies were numerous in the nucleus preopticus medialis and lateralis, nucleus anterior, nucleus ventromedialis and dorsomedialis, nucleus lateralis, nucleus arcuatus, nucleus preamillaris ventralis and dorsalis. Only occasional cell bodies were BN/GRP-IR positive in these areas. The results indicate that the neuronal systems in the hypothalamus containing BN/GRP-IR and SP-IR are separate, though the terminal fields in many areas overlap. The column chromatography also clearly demonstrated that SP and BN/GRP are two separate neuropeptides. Biochemical analysis of BN/GRP-IR revealed that BN/GRP-IR is composed of GRP(1-27) and GRP(18-27), moreover bombesin is not present in rat CNS.

The opioid peptide, met⁵-enkephalin-arg⁶-gly⁷-leu⁵ (YGGFMRFGGL) content is higher in the dorsal horn than the ventral horn of rat spinal cord. YGGFMRFGGL containing neuronal cell bodies were detected in laminae I-V. They were most dense in laminae I. Cell bodies were also seen around the central canal. In the ventral horn, the antiserum against YGGFMRFGGL stained only fibers and terminals, no cell bodies were stained. The dorsal lateral fasciculus was stained with the YGGFMRGL antiserum.

The high concentrations of YGGFMRGL in laminae I and II of dorsal horn suggests a role for this peptide in controlling or modulating spinal reflexes. The possible association of YGGFMRGL with nociceptive afferent input can now be better investigated based on this study.

The exact localization and chemical nature of BN/GRP-IR needs clarification to proceed in the investigation of the role of this peptide in neuronal function.

Publications:

Panula, P., Hadjiconstantinou, M., Yang, H.-Y.T., and Costa, E.: Immunohistochemical localization of bombesin/gastrin-releasing peptide and substance P in primary sensory neurons. J. Neurosci. 3: 2021-2029, 1983.

Panula, P., Cosenza-Murphy, D., Yang, H.-Y.T., and Costa, E.: Binding of GRP(14-27) but not bombesin or GRP(1-27) to hypothalamic magnocellular elements: An immunohistochemical study. J. Histochem. Cytochem. 32: 202-208, 1984.

Panula, P., Yang, H.-Y.T., and Costa, E.: Coexistence of Met⁵-enkephalin-Arg⁶-Phe⁷ with Met⁵-enkephalin and the possible role of Met⁵-enkephalin-Arg⁶-Phe⁷ in neuronal function. In Chan-Palay, V., and Palay, S.L. (Eds.): Coexistence of

Neuroactive Substances in Neurons. New York, John Wiley and Sons, Inc., 1984, pp. 113-126.

Panula, P., Yang, H.-Y.T., and Costa, E.: Histamine-containing neurons in the rat hypothalamus. Proc. Natl. Acad. Sci. USA 81: 2572-2576, 1984.

Panula, P., Yang, H.-Y.T., and Costa, E.: Comparative distribution of bombesin/GRP- and substance-P-like immunoreactivities in rat hypothalamus. J. Comp. Neurol. 224: 606-617, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
 NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
 Z01 MH 01559-03 SMRP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Met-Enkephalin-Arg-Phe and Phe-Met-Arg-Phe-NH₂ in the Brain and Spinal Cord

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	H.-Y.T. Yang	Section Chief	SMRP	NIMH
Others:	J. Chou	Visiting Fellow	SMRP	NIMH
	J. Tang	Visiting Fellow	SMRP	NIMH
	E. Costa	Lab Chief	SMRP	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Neuropeptide Section

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	1.3	0

CHECK APPROPRIATE BOX(ES)

(a) Human subjects
 (a1) Minors
 (a2) Interviews
 (b) Human tissues
 (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Previously, possible interaction between the opioid peptide derived from proenkephalin met-enkephalin-arg-phe (YGGFMRF) and phe-met-arg-phe-NH₂ (FMRF-NH₂) was investigated. FMRF-NH₂ injected intrathecally reduced the analgesia induced by YGGFMRF. Though FMRF-NH₂ immunoreactive material (FMRF-NH₂-IR) is present in rat spinal cords, it is different from FMRF-NH₂. Because of this, endogenous FMRF-NH₂-IR was isolated from the medulla oblongata of bovine brain by immunoaffinity column chromatography using IgG from FMRF-NH₂ antiserum conjugated to Sepharose 4B as affinity ligand. The partially purified material, which contained mainly 2 immunoreactive peptides with molecular weight of about 1500-1800 daltons, attenuated the analgesia induced by YGGFMRF when injected intrathecally. The results suggest a naloxone like activity for FMRF-NH₂ or endogenous FMRF-NH₂-IR. Physiological role of endogenous FMRF-NH₂-IR was assessed using IgG from FMRF-NH₂ antiserum as antagonist. IgG from FMRF-NH₂ antiserum caused a moderate long lasting naloxone reversible analgesia. This result implies a modulatory role for FMRF-NH₂-IR in analgesia possibly mediated by endogenous opioid peptide. FMRF-NH₂-IR is unevenly distributed in the rat brain with high concentrations in pituitary and hypothalamus and low levels in hippocampus and cerebellum. Biochemical analysis revealed three different forms of FMRF-NH₂-IR in the rat brain and, interestingly, the proportion of the 3 FMRF-NH₂-IR varies greatly in different regions of rat brain. Whether all three different forms have similar biological activities still remains to be established.

Project Description:

Met⁵-enk-arg⁶-phe⁷ (YGGFMRF) is structurally similar to the neuropeptide phe-met-arg-phe-NH₂ (FMRF-NH₂). Interestingly, in molluscan tissue, FMRF-NH₂ was reported to have some biological activities which are opposite to those of the opioid peptides of the enkephalin family. Though, FMRF-NH₂ is a neuropeptide of clam origin (Greenberg et al., *Fed. Proc.* 42: 82-86, 1983), FMRF-NH₂ immunoreactive material (FMRF-NH₂-IR) is present in mammalian CNS. We have previously found that both YGGFMRF and FMRF-NH₂-IR are concentrated in the dorsal horn of rat spinal cord. In searching for a biological role of these two structurally similar neuropeptides, possible interaction between FMRF-NH₂-IR and YGGFMRF was studied. YGGFMRF injected intrathecally along with peptidase inhibitors, bestatin and captopril, was found to induce a moderate analgesia and this analgesia was significantly reduced if rats were preinjected intrathecally with FMRF-NH₂. Biochemical analysis of FMRF-NH₂-IR in rat spinal cord revealed 3 different molecular forms of FMRF-NH₂ and all of them are different from authentic FMRF-NH₂. The molecular weights of the 3 endogenous FMRF-NH₂-IR were estimated by BioGel P-2 column to be around 1500-1800 daltons. Since endogenous FMRF-NH₂-IR differs from FMRF-NH₂, which is a neuropeptide of clam origin, the mammalian FMRF-NH₂-IR was purified from bovine medulla oblongata by immunoaffinity column chromatography. For the immunoaffinity ligand, IgG was purified from FMRF-NH₂ antiserum and then conjugated to CNBr activated Sepharose-4B. The FMRF-NH₂-IR purified by this affinity column chromatography was found to contain two main FMRF-NH₂-IR peptides with molecular weight of about 1500-1800 daltons but not YGGFMRF. These two mammalian FMRF-NH₂-IR, when injected intrathecally prior to YGGFMRF, reduce the analgesia induced by the opioid heptapeptide. The results suggest a naloxone like biological activity for FMRF-NH₂ and mammalian FMRF-NH₂-IR. In order to assess the physiological relevance of this interaction between injected YGGFMRF and FMRF-NH₂ or FMRF-NH₂-IR, injections of IgG prepared from FMRF-NH₂ antiserum were used in an attempt to block the antioiopic activity of endogenous FMRF-NH₂-IR. Intraventricular injections of IgG from FMRF-NH₂ antiserum but not that from control serum was found to cause a long-lasting and naloxone reversible moderate analgesia. The results suggest an important role for endogenous FMRF-NH₂-IR in modulating the analgesia which may be mediated by endogenous opioid peptides such as enkephalins.

Recently whether mammalian FMRF-NH₂ is identical with δ_1 -MSH, NPY, PYY or APP has been considered by various investigators. Because of this, in further searching for the biological role of FMRF-NH₂-IR, we have decided to characterize the FMRF-NH₂-IR in various rat brain regions. The distribution of FMRF-NH₂-IR (fmol/mg prot) in various rat brain regions is cerebellum (5.4±0.7); medulla oblongata (16±1.3); striatum (5.4±0.6); midbrain (13±1.4); hippocampus (3.5±0.5); hypothalamus (35±3.6); cortex (4.7±0.7); pituitary (10±11); spinal cord (40±2.7). The brain parts and pituitaries were dissected from rats killed by focused microwave irradiation and spinal cord was dissected from decapitated rats. The values were calculated from the standard curve prepared with FMRF-NH₂ and are expressed in arbitrary units calculated in FMRF-NH₂ equivalents. Characterization of FMRF-NH₂-IR by HPLC revealed that there were 3 main immunoreactive peptides with retention times of 28 min (A), 32 min (B) and 40 min (C) and all of them are different from FMRF-NH₂, δ_1 -MSH, NPY. Interestingly, the proportion of the three FMRF-NH₂-IR varies greatly in different brain regions. Content of C form is high in spinal cord and medulla oblongata and low in pituitary and hypothalamus. The midbrain contains the 3 forms in almost equal proportion. The results in this study suggest

that the biological role of mammalian FMRF-NH₂-IR may not be easily explored by using synthetic FMRF-NH₂ and possible different biological property of various form should be considered.

The significance of this study on biomedical research is that the endogenous antiopiate, FMRF-NH₂-IR, may open up a new direction for studying tolerance to opiate analgesia.

The proposed course of this study is to purify the different forms of FMRF-NH₂-IR and determine their sequence and synthesize the new structure to obtain specific antibody.

Publications:

Del Rio, J., Naranjo, J.R., Yang, H.-Y.T., and Costa, E.: Substance P-induced release of met⁵-enkephalin from striatal and periaqueductal gray slices. Brain Res. 279: 121-126, 1983.

Chou, J., Tang, J., Yang, H.-Y.T., and Costa, E.: Increase of striatal met⁵-enkephalin-arg⁸-phe⁷ (YGGFMRF) content elicited by long-term treatment with haloperidol. J. Pharmacol. Exp. Ther. 229: 171-174, 1984.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 01560-03 SMRP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Met5-enkephalin-arg6-phe7 in Peripheral Tissue

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	J. Tang	Visiting Fellow	SMRP	NIMH
Others:	J. Chou	Visiting Fellow	SMRP	NIMH
	P. Panula	Visiting Fellow	SMRP	NIMH
	H.-Y.T Yang	Section Chief	SMRP	NIMH
	E. Costa	Lab Chief	SMRP	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Neuropeptide Section

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MAN-YEARS:

0.7

PROFESSIONAL:

0.7

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The presence of neuropeptides as well as peptide hormones in lung has been well established. Previously we have detected met5-enkephalin-arg6-phe7 (ME-arg-phe), bombesin and substance P-like peptides in the rat lung by specific radioimmunoassays. In this study, the cellular locations of these peptides were investigated by immunohistochemical technique. Bombesin and substance P immunoactivities were found in nerve fibers present in bronchi and blood vessels while ME-arg-phe immunoactivity was localized in small APUD-like cells around the wall of some bronchi. Because of its cellular location, the release of ME-arg-phe from lung slices was studied. Superfusion of lung cubes with Krebs's solution containing substance P or bombesin shows that ME-arg-phe can be released in a Ca⁺⁺ dependent manner. The results seem to suggest that when substance P and bombesin are released by nerve impulses from their storage site in nerve fibers, they can facilitate or even trigger the release of ME-arg-phe directly or indirectly.

Proposed Course:

This project has been terminated.

Publications:

Tang, J., Chou, J., Yang, H.-Y.T.⁷ and Costa, E.: Substance P stimulates the release of met⁵-enkephalin-arg⁶-phe⁷ and met⁵-enkephalin from rat spinal cord. Neuropharmacology 22: 1147-1150, 1983.

Chou, J., Tang, J., and Costa, E.: Met⁵-enkephalin-arg⁶-phe⁷ content of human and rabbit plasma. Life Sci. 32: 2589-2595, 1983.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 01561-03 SMRP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cholecystokinin in Brain

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: M.J. Iadarola

PRAT Fellow

SMRP

NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Neuropeptide Section

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MAN-YEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project is concerned with two neuropeptides, cholecystokinin (CCK) and met5-enkephalin-arg6-gly7-leu8 (YGGFMRGL) and an attempt to evaluate whether in the spinal cord, these two peptides are involved in sensory processing, in particular pain; in fact, YGGFMRGL injection in the subarachnoid space suppresses pain sensation whereas CCK blocks this action. In spinal cord, the highest levels of YGGFMRGL and CCK are found in laminae I and II, respectively. Two molecular weight forms of YGGFMRGL were found in the spinal cord, the first is identical to authentic YGGFMRGL; the second is a 5.3 Kd N-terminal extended form. Release was studied with an in vivo perfusion system of the subarachnoid space of the cord coupled with bilateral electrical stimulation of the sciatic nerves. High and low molecular weight forms which are present in spinal cord are released upon stimulation suggesting both forms participate in neuromodulation. A model of chronic inflammation using hindpaw injections of Freund's adjuvant is being studied as an attempt to relate pain with changes in the dynamic state of endogenous CCK, YGGFMRGL and dynorphin. Profound steady state changes of dynorphin occur in the dorsal horn to which the sensory fibers of the affected limb project. CCK and other peptides that block the analgesic action of opiates are also being examined in this condition. These studies on spinal cord are relevant to sensory processing, in particular pain, and may be important in understanding CNS neurochemical changes due to inflammation and other chronic pain states.

Project Description:

The project is centered around the interrelationships between CCK- and opioid peptide-containing neurons in brain and spinal cord. These interactions are examined by measuring changes in small and large molecular weight peptide content, peptide release and content of precursor mRNA with cloned cDNA probes.

In spinal cord, met⁵-enkephalin-arg⁶-gly⁷-leu⁸ (YGGFMRGL) is concentrated in the dorsal horns at all spinal segments, 3-fold lower levels are found in ventral cord. Immunocytochemical analysis revealed numerous cell bodies and nerve terminals in dorsal cord (laminae I, II and IV); only nerve terminals were observed in ventral cord. Terminals in ventral cord appeared to surround perikarya of motor neurons suggesting a role in spinal reflexes. Sacral cord had an additional cell group dorsal to the central canal which densely innervated the sacral parasympathetic preganglionic nucleus. This additional cell group probably contributes to the high levels of YGGFMRGL found in this spinal region.

Gel filtration and HPLC analysis of spinal cord extracts disclosed two major immunoreactive molecules: one was YGGFMRGL, the other was a high molecular weight (HMW) N-terminal extended form of about 5.3 kilodaltons. This HMW form accounted for about 30-50% of the total immunoreactivity and was found in both ventral and dorsal cord. Studies were conducted to determine whether these materials were released by drug injections or electrical stimulation of afferent axons. Perfusion of the subarachnoid space *in vivo* and analysis of the perfusate was performed during bilateral electrical stimulation of the sciatic nerve or inclusion of substance P in the perfusate. Low and HMW-forms of YGGFMRGL were released by both types of stimuli. Recovery of the HMW-form was observed most consistently, proportionally 10-50% of the released immunoreactivity was recovered as YGGFMRGL despite its higher content in tissue. We attribute this to inadequate protection of the released YGGFMRGL from enzymatic breakdown by tissue peptidases.

Future studies will try to determine which class of peptidases (amino-, endo-, or dipeptidylcarboxypeptidases) are important in YGGFMRGL degradation, *in vivo*. Inclusion of bestatin, an aminopeptidase inhibitor, and phosphoramidon an inhibitor of metalloendopeptidases (both at 10^{-5} M) in the perfusate appears to increase the recovery of released YGGFMRGL by at least 50%. We shall also examine the effect of these inhibitors on *in vitro* release from slices of cord where conditions can be more closely controlled. Optimization of conditions for release is important and forms the basis from which the actions of drugs and other peptides such as CCK on release can be evaluated. Modulation of spinal opiate peptide systems in spinal cord dorsal horn by an alteration in sensory state is being examined with a model of chronic inflammation. Injection of Freund's adjuvant into the hindpaw produces swelling and inflammation of the foot. Analysis of peptide levels in dorsal horn ipsilateral to the affected limb revealed an increase (116%) in dynorphin compared to the contralateral dorsal horn. Alteration in YGGFMRGL content will be studied as well as FMRF amide, CCK and substance P in this model system.

The significance to biochemical research is these studies will increase our understanding of the neurochemical basis of sensory processing, in particular pain, and the CNS neurochemical changes due to inflammation and other chronic pain states.

Publications:

Kleinman, J.E., Iadarola, M.J., Govoni, S., Hong, J., Gillin, J.C., and Wyatt, R.J.: Post-mortem measurements of neuropeptides in human brain. Psychopharmacol. Bull. 19: 375-377, 1983.

Majane, E.A., Iadarola, M.J., and Yang, H.-Y.T.: Distribution of met⁵-enkephalin-arg⁶-phe⁷ in rat spinal cord. Brain Res. 264: 336-339, 1983.

Iadarola, M.J., and Yang, H.-Y.T.: Relationship between cholecystokinin in rat forebrain: A biochemical study of co-localization. J. Neurochem., in press.

Iadarola, M.J., Panula, P., Majane, E.A., and Yang, H.-Y.T.: The opioid octapeptide met⁵-enkephalin-arg⁶-gly⁷-leu⁸: Characterization and distribution in rat spinal cord. Brain Res., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

701 MH 01562-03 SMRP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Cholinergic Neuronal System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: M. Hadjiconstantinou Guest Researcher SMRP NIMH

Others: J.L. Meek Pharmacologist SMRP NIMH
N.H. Neff Section Chief SMRP NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Biochemical Pharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MAN-YEARS:

0.3

PROFESSIONAL:

0.3

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The purpose of this project is to identify and study the acetylcholine-containing neurons. Our present objective is to improve our existing method for assaying acetylcholine and to study the consequences of chronic treatment with diisopropyl-fluorophosphate, an irreversible cholinesterase inhibitor, on the brain content of acetylcholine and content and turnover of dopamine.

Project Description:

We have developed a simple method for assaying choline and acetylcholine in neuronal tissue (Z01 MH 01562-01 SMRP). Our present goal was: 1) to determine whether our analytical procedure might be used to study the rate of formation of acetylcholine in regions of brain and 2) to study the consequence of chronic treatment with diisopropylfluorophosphate (DFP) on brain acetylcholine and dopamine content.

1. With our previously published analytical procedure, choline and acetylcholine are extracted and separated by reverse phase HPLC. Choline and acetylcholine are converted to hydrogen peroxide by continually pumping and mixing acetylcholinesterase and choline oxidase with the column effluent. The electrochemical detection of hydrogen peroxide is a quantitative measure of acetylcholine and choline. Currently, we bind the enzymes to a short anionic column, thus eliminating the need for a second pump and the continual waste of enzymes. To study the turnover rate of acetylcholine, methyl-³H-choline is injected intravenously into mice. The extraction and assay are carried out as usual but the fractions corresponding to the detector response for choline and acetylcholine are collected for the measurement of radioactivity. In this way specific radioactivity of endogenous choline and acetylcholine are estimated in the same sample allowing us to estimate acetylcholine turnover rate.
2. Following the administration of a single dose of DFP, an irreversible cholinesterase inhibitor, there is a rise of acetylcholine in the rat striatum and frontal cortex. With chronic treatment, striatal acetylcholine content returns to normal, but frontal cortex acetylcholine remains elevated. In striatum but not frontal cortex, there is a rise of dopamine content and turnover after chronic DFP treatment. We speculate that dopamine content and turnover are increased after chronic DFP because the nigrostriatal neuronal feedback loop is activated to compensate for increased cholinergic tone.

Acetylcholine was the first neurotransmitter to be described. It has not been investigated to the same extent as the other transmitters because there has not been a simple universal method for its analysis. Our simple analytical procedure should allow for an increased research effort into brain cholinergic systems. Our initial studies have focussed on the role that cholinergic neurons have on regulating the synthesis and release of dopamine in cortex and striatum. Although it has been suspected that cholinergic neurons in the striatum could modulate the synthesis and storage of dopamine in striatum our studies are the first to provide direct experimental evidence. Moreover, we found that frontal cortex and striatum are apparently not modulated by the same neuronal control mechanisms. This is particularly important now that several brain disorders are associated with a deficiency of cholinergic neurons in limited regions of brain, i.e., Alzheimer's disease. Our goal is to provide new insight into these problems.

The research will be terminated and the work prepared for publication.

Publications:

Potter, P.E., Meek, J.L., and Neff, N.H.: Acetylcholine and choline in neuronal tissue measured by HPLC with electrochemical detection. J. Neurochem. 41: 188-194, 1983.

Olianas, M.C., Onali, P., Neff, N.H., and Costa, E.: Muscarinic receptors modulate dopamine-activated adenylate cyclase of rat striatum. J. Neurochem. 41: 1364-1369, 1983.

Hadjiconstantinou, M., and Neff, N.H.: Ascorbic acid could be hazardous to your experiments: A commentary on dopamine receptor binding sites with speculation on a role for ascorbic acid in neuronal function. Neuropharmacology 22: 939-943, 1983.

Olianas, M.C., Onali, P., Schwartz, J.P., Neff, N.H., and Costa, E.: The muscarinic receptor adenylate cyclase complex of rat striatum: Desensitization following chronic inhibition of acetylcholinesterase activity. J. Neurochem. 42: 1439-1443, 1984.

Potter, P.E., Hadjiconstantinou, M., Meek, J.L., and Neff, N.H.: Measurement of acetylcholine turnover rate in brain: An adjunct to a simple HPLC method for choline and acetylcholine. J. Neurochem., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 MH 01563-03 SMRP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Adenosine and GABA-B Receptor Interactions

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	W. Wojcik	Guest Researcher	SMRP	NIMH
Others:	D. Cavalla	Visiting Fellow	SMRP	NIMH
	N.H. Neff	Section Chief	SMRP	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Biochemical Pharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MAN-YEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is to determine if adenosine A1 and GABA-B receptors, both of which are negatively coupled to adenylate cyclase, interact when they are associated with the same neuronal cells. Our present objective is to determine if the nonadditive responses to stimulation of adenosine A1 and GABA-B receptors in striatum and cerebellum are the result of both receptors being found on the same cell type and perhaps coupled to the same cyclase catalytic unit.

Project Description:

Both the adenosine A1 and the GABA-B receptors are coupled to the inhibitory adenylate cyclase system of plasma membranes. Recent evidence have shown many similarities between receptors which are inhibitory to cyclase and various biochemical, electrophysiological and behavioral responses. This class of receptors are believed to be present on excitatory (eg. glutamatergic, aspartergic and cholinergic) nerve terminals, inhibiting the release of the neurotransmitter. This effect is supported by the reduced excitatory postsynaptic potentials observed with intracellular recording techniques. This class of receptors are also shown to directly hyperpolarize the cell. Behaviorally, the agonists have anticonvulsant and sedative activity and are useful in attenuating the morphine withdrawal symptom. These observations lead us to hypothesize that the adenosine A1 and the GABA-B receptors are associated with the same cell and that an interaction may occur on the receptor-mediated responses to cyclase. Our objectives were: 1. to determine whether an interaction occurs between the A1 and GABA-B receptor response to cyclase from various rat brain regions; 2. to determine which cell type and/or pathway are these two receptors associated and 3. to determine the mechanism of the interaction to cyclase.

1. We have previously shown that the stimulation of the A1 and the GABA-B receptors in rat cerebellum results in a nonadditive, converging inhibitory response to cyclase. Nonadditivity to cyclase with PIA and baclofen, agonists at the A1 and GABA-B receptors, respectively, is also found in the striatum. There does not appear to be any interaction between the adenosine A2 receptors which stimulate adenylate cyclase activity and the GABA-B receptors in the striatum.
2. Since we have determined that the nonadditive response to cyclase with PIA and baclofen results from both receptors being associated with the same cerebellar cell type, the granule cell, we also performed various striatal lesions to locate the cells or terminals which contain the GABA-B receptors. Three striatal lesions were performed to determine if the GABA-B receptor is associated with the cortical-striatal terminals, the nigral-striatal dopaminergic terminals or the intrinsic, kainic acid sensitive, neurons. Our results show the GABA-B receptor is associated with the cortical-striatal terminals and the intrinsic neurons. These same lesions also resulted in a loss of adenosine A1 receptors. Thus, we may suggest both receptors are associated with the same cells and would further support our hypothesis that nonadditive receptor-mediated responses represent receptors interacting with the cyclase system on the same membrane.
3. We proposed that the nonadditive response seen in the cerebellar membranes with PIA and baclofen may result from an interaction occurring at any of three levels: at the receptors or at the level of the inhibitory guanine nucleotide units or at the catalytic units. Our results with ligand binding to the A1 site in the presence of baclofen and the PIA-stimulation of GTPase activity (which reflects the Ni unit) in the presence of baclofen, shows both receptors are capable of transmitting their "signal" to the Ni unit without any impairment. However, the "signal" is attenuated at the catalytic unit which is seen as a nonadditive response on cyclase activity. Since this nonadditivity is also observed in the presence of forskolin-stimulation of cyclase activity,

we conclude that the nonadditivity results from a limited number of catalytic units. Thus under maximal receptor occupancy with PIA and baclofen, there are not enough catalytic units to interact with and a converging response arises.

Present studies attempt to use the clonal, NG108-15, cell line as a model system for studying the interactions between receptors which are inhibitory to adenylate cyclase. These cells have the cholinergic muscarinic, opiate delta and adrenergic alpha-2 receptors which inhibit cyclase. Furthermore, primary cultures of cerebellar granule cells are currently being used to study the adenosine A1 and GABA-B receptors.

Some investigators have suggested that anxiolytic drugs act at adenosine receptor sites. At present, however, the role of adenosine in brain function is unclear. Our studies are providing basic information needed to evaluate adenosine's role in brain physiology. They also demonstrate that endogenous substances that inhibit adenylate cyclase activity may act at specific receptor but may utilize the same post recognition site elements to reduce cyclase activity. Indeed, drugs acting at separate and specific receptors could produce the same pharmacological response in a tissue by utilizing the mechanism we have unmasked. Our studies of brain receptor mechanisms may provide a clue to the functional role of both adenosine and GABA-B neuronal systems.

The research will be terminated and the studies prepared for publication.

Publications:

Wojcik, W.J., and Neff, N.H.: Adenosine A₁ receptors are associated with cerebellar granule cells. J. Neurochem. 41: 759-763, 1983.

Wojcik, W.J., and Neff, N.H.: Location of adenosine release and adenosine A₂ receptors to rat striatal neurons. Life Sci. 33: 755-763, 1983.

Wojcik, W.J., and Neff, N.H.: Differential location of adenosine A1 and A2 receptors in striatum. Neurosci. Lett. 41: 55-60, 1983.

Wojcik, W.J., and Neff, N.H.: Baclofen stimulates GABA-B receptors to inhibit adenylate cyclase activity in brain. Mol. Pharmacol. 25: 24-28, 1984.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 01565-03 SMRP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of GABA-A and GABA-B Receptor Function

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	M.D. Majewska	Visiting Fellow	SMRP	NIMH
Others:	D.M. Chuang	Chemist	SMRP	NIMH
	E. Costa	Lab Chief	SMRP	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Monoclonal Antibody Group

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

1.3	1.3	0
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CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This study concerns an investigation of the role of Ca^{2+} in the regulation of GABA binding to GABA-A and GABA-B recognition sites located in the synaptic membranes of rat brain. At 37° the binding of 3H-GABA to GABA-B recognition sites is dramatically stimulated by Ca^{2+} with a $K_a \approx 10^{-5}M$ while the binding to GABA-A recognition sites is only slightly but significantly enhanced by Ca^{2+} with a $K_a \approx 5 \times 10^{-7}M$. The Ca^{2+} effect on GABA-A recognition sites is temperature dependent and requires calmodulin but involves neither phospholipase A2 nor Ca^{2+} -dependent proteases. Only GABA-A recognition sites are linked to benzodiazepine recognition sites and also this interaction is modulated by Ca^{2+} at physiological ion concentrations. Diazepam and low μM Ca^{2+} cause the appearance of a high affinity binding site for GABA-A recognition sites. The number of GABA-B recognition sites measured at 37° is about 70% higher than that measured at 4° . This temperature-dependent increase in the number of GABA-B recognition sites is calmodulin independent, probably Ca^{2+} -dependent proteases are operative. We have also studied whether GABA-A and benzodiazepines recognition sites located on C6-glioma and neuroblastoma NB2a cells are linked to some other transducer, for instance phospholipase A2. In C6-glioma but not NB2a cells, prelabeled with 14C-arachidonic acid, a GABA agonist muscimol stimulates the release of 14C-arachidonic acid. This increased release is due to an activation of phospholipase A2. This process is blocked by bicuculline, a classical GABA-A receptor blocker but not by inhibitors of Cl^{-} channel or GABA uptake blockers. The phospholipase A2 activation by muscimol is potentiated by several benzodiazepines, but not by clonazepam. Analyses of the radioactive metabolites released by HPLC reveal that a substantial amount of prostaglandin D2 is formed when muscimol is supplemented with diazepam. Prostaglandin D2 may be a neuromodulator which serves as a linkage of glial cells to neuronal function. We are currently investigating the possibility of the involvement of glial benzodiazepine receptors in the regulation of GABA function mediated through the formation of certain classes of prostaglandins.

1147

Proposed Course:

This project has been terminated.

Publication:

Majewska, M.D., and Chuang, D.M.: Modulation by calcium of γ -aminobutyric acid binding to GABA_A and GABA_B recognition sites in rat brain: Involvement of different mechanisms. Mol. Pharmacol. 25: 352-359, 1984.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 01566-03 SMRP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Mechanisms in the Antidepressant Action of (-)Deprenyl

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	O. Gandolfi	Guest Researcher	SMRP	NIMH
Others:	M.L. Barbaccia	Guest Researcher	SMRP	NIMH
	E. Costa	Lab Chief	SMRP	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Molecular Neurobiology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MAN-YEARS:

0.5

PROFESSIONAL:

0.5

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

(-)Deprenyl is a selective inhibitor of monoamine oxidase (MAO) type B. MAO-B uses as preferential substrates phenylethylamine and dopamine, while MAO-A preferentially catabolizes norepinephrine (NE), dopamine and serotonin (5HT). At a dose of 5 mg per day, which selectively inhibits the MAO-B, (-)deprenyl relieved many symptoms of depressed patients without eliciting the strong side-effects induced by other MAO inhibitors. In rats receiving (-)deprenyl (1 μ mol/kg, s.c., for 21 days) the NE-stimulated cAMP accumulation and the β -adrenergic receptor number in minces and crude synaptic membranes from frontal cortex were significantly decreased. Moreover after the same treatment schedule the number of 3H-imipramine recognition sites in frontal cortex and hippocampus was significantly increased. Interestingly, (-)deprenyl is not active "in vitro" as a displacer of 3H-imipramine specifically bound to rat brain membranes, and the possibility that this effect is due to the formation of one of the (-)deprenyl metabolites, amphetamine, or to the blockade of MAO-B, was ruled out. A selective lesion of the 5HT axon terminals prevented the increase of 3H-imipramine binding and the attenuation of the β -adrenergic-coupled cAMP generating system evinced by repeated injections of (-)deprenyl. These results suggest that many of the (-)deprenyl are typical of the antidepressant actions and that a primary modification of the serotonergic neurons can determine the attenuation of signal amplification at noradrenergic receptors, believed to mediate the relief of symptoms of affective disorders.

Proposed Course:

This project has been terminated.

Publications:

Zsilla, G., Barbaccia, M.L., Gandolfi, O., Knoll, J., and Costa, E.: (-)Deprenyl a selective MAO "B" inhibitor increases ³H-imipramine binding and decreases β -adrenergic receptor function. Eur. J. Pharmacol. 89: 111-117, 1983.

Gandolfi, O., Barbaccia, M.L., and Costa, E.: The (-)deprenyl action on β -adrenergic receptors require the integrity of brain serotonergic axon terminals. Eur. J. Pharmacol. 100: 233-237, 1984.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 01567-02 SMRP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Role of Synaptosomal Basic Proteins in the Control of GABA Receptor Function

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	F. Vaccarino	Guest Researcher	SMRP	NIMH
Others:	A. Guidotti	Section Chief	SMRP	NIMH
	E. Costa	Lab Chief	SMRP	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Neuroendocrinology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MAN-YEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

GABA-modulin (GM), a basic protein operative in mediating GABA benzodiazepine receptor interaction, was present in high concentrations in association with GABA recognition sites in rat brain synaptosomes. GM is basic in nature and is rich in arginine and lysine residues. The above characteristics link this peptide to the general class of brain basic peptides. However physiochemical and biochemical observations indicate that GM is different from any other known basic peptide. This difference has been confirmed by using a monoclonal antibody which reacts with GM, but not with any other basic brain protein. With this antibody, it is possible to demonstrate the presence of high concentrations of GM in neurons rich in GABA receptors.

Project Description:

Binding of GABA to specific recognition sites on postsynaptic neuronal membranes is regulated by a peptide termed GABA-modulin (GM). GM purified from rat brain and added in vitro to crude synaptic membranes, noncompetitively reduces the number of high affinity GABA recognition sites. GM has been extracted and purified from the synaptosomal fraction of rat brain where it represents approximately 0.5% of the total synaptosomal protein. GM shares a number of characteristics with the class of highly basic proteins isolated from myelin and, in particular, with the rat small myelin basic protein (SMBP). However unlike SMBP, GM is selectively located in synaptosomes and differs from SMBP in its amino acid composition (it contains more Gly and Lys and less Arg residues) and in molecular weight (17000 and 15000 daltons for GM and SMBP, respectively). Synaptosomal GM fails to bind ^3H -muscimol, but noncompetitively inhibits the binding of ^3H -muscimol to purified synaptic membrane with an IC_{50} of $0.5 \mu\text{M}$. Cyanogen bromide treatment generates a major 13,000 MW fragment from both SMBP and GM. These two fragments showed differences in the amino acid composition and sequence. Moreover, maps of the peptide fragments generated from GM and SMBP by partial proteolysis with trypsin and the staphylococcal V8 protease were different.

Since polyclonal antibodies raised against GM cross-react with myelin basic proteins, monoclonal antibodies have been prepared against GM in order to study the physiological role and localization of this protein in brain. NZB/N mice have been immunized and their lymphocytes fused with P3x63 myeloma cells. One of the cloned hybrids was selected because it secreted antibodies specific for GM, failed to cross-react with a 100-fold higher concentration of SMBP, or with large myelin basic protein. These antibodies have been used to detect GM in a population of neurons particularly rich in GABA-receptors, the cerebellar granule cells. Granule cells in primary culture are not myelinated and contain less than 5% of the astrocyte and the oligodendrocyte cells. These primary cultures represent an excellent model for studying the GABA-BZ- Cl^- receptor/ionophore complex and its regulation. They express in their membrane GABA receptors, BZ recognition sites, as well as chemically modulated Cl^- channel. Recognition sites for ^3H -muscimol, ^3H -flunitrazepam and ^3H -t-butylbicyclophosphorothionate can be studied under physiological conditions and in undisrupted cells. Granule cells contain a protein that when purified by a standard procedure, is identical in molecular weight and amino acid composition as GM purified from all brain synaptosomes. This protein also reacts with our specific monoclonal antibody and its concentration in the granule cells is $0.52 \mu\text{g}/\text{mg}$ protein.

The presence of GM in a homogeneous population of granule cells which expresses GABA receptors, receives a strong GABA input, and does not use GABA as a neurotransmitter, is highly indicative of a postsynaptic localization for this protein. Moreover, the availability of a monoclonal antibody probe could be very important in studying the functional role of GM in association with GABA-receptors. We believe that a more detailed study on the mode by which GM controls GABA receptor function may lead to new models to characterize the molecular mechanism whereby potent and specific drugs, such as benzodiazepines and beta-carbolines, facilitate or inhibit the number of GABA recognition sites available.

Publications:

Guidotti, A., Corda, M.G., Wise, B.C., Vaccarino, F., and Costa, E.: GABAergic synapses. Neuropharmacology 22: 1471-1479, 1983.

Vaccarino, F., Conti-Tronconi, B.M., Panula, P., Guidotti, A., and Costa, E.: GABA-modulin: A synaptosomal basic protein that differs from small myelin basic protein of rat brain. J. Neurochem., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
 NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01568-02 SMRP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Sulfation of Cholecystokinin in Brain

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	J.L. Meek	Pharmacologist	SMRP	NIMH
Others:	O. Giorgi	Visiting Fellow	SMRP	NIMH
	K.L. Kirk	Research Chemist	LC	NIADDKD

COOPERATING UNITS (if any)

Laboratory of Chemistry, Natl. Inst. Arthritis, Diabetes, and Digestive and Kidney Diseases, Bethesda, Md.

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

High Pressure Liquid Chromatography Group

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MAN-YEARS

0.1

PROFESSIONAL:

0.1

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The object of this study was to determine whether: 1) the sulfation of cholecystokinin octapeptide (CCK) could be demonstrated in brain; 2) whether the rate of CCK synthesis could be calculated using sulfate incorporation as a method; 3) whether inhibitors could be found which would permit a pharmacological assessment of CCK turnover and the physiological role of CCK in brain cortex. Goals 1 and 2 were fully met during the previous fiscal year, and the data were summarized for publication. Goal 3 was approached via study of several compounds obtained from commercial sources or synthesized by Dr. K.L. Kirk of NIADDKD. None of the compounds tested were able to completely inhibit sulfation at doses which were not toxic. These data have been accepted for publication.

Project Description and Proposed Course:

The experiments performed previously for this project were summarized for publication and this project was terminated. During the course of the HPLC work involved in this study, a useful "shortcut" was discovered which will be published as a short note in Anal. Chem.

Publications:

Meek, J.L., Iadarola, M.J., and Giorgi, O.: Cholecystokinin turnover in brain. Brain Res. 276: 375-378, 1983.

Giorgi, O., and Meek, J.L.: Sulfation of peptides and simple phenols by rat brain phenolsulfotransferase: Inhibition by dichloronitrophenol. Biochem. Pharmacol., in press.

Meek, J.L.: Teflon tubing as a removable replacement for steel ferrules in HPLC. Anal. Chem., in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 01569-02 SMRP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Interaction with Neuropeptides

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	J. Tang	Visiting Fellow	SMRP	NIMH
Others:	H.-Y.T. Yang	Section Chief	SMRP	NIMH
	M.J. Iadarola	PRAT Fellow	SMRP	NIMH
	W. Fratta	Visiting Scientist	SMRP	NIMH
	E. Costa	Lab Chief	SMRP	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Neuropeptide Section

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MAN-YEARS:

0.9

PROFESSIONAL:

0.9

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

It was previously demonstrated that antinociception elicited by morphine can be reduced by either FMRF-NH2, injected intraventricularly or intrathecally, or CCK-8-SO4, injected intrathecally. Proglumide, a CCK receptor blocker, antagonized the antiopiate activity of CCK-8-SO4 as expected and surprisingly also blocked the antiopiate activity of FMRF-NH2. The naturally occurring FMRF-NH2 immunoreactive material is different from authentic FMRF-NH2. In order to assess the role of endogenous FMRF-NH2 immunoreactivity and CCK-8-SO4 in opiate action, the effects of proglumide and FMRF-NH2 antibody on opiate antinociception was studied. Both proglumide and IgG, isolated from FMRF-NH2 antiserum, were found to potentiate the morphine analgesia and also lessen the development of acute morphine tolerance induced by repeated injections of morphine at 2 hour intervals in rats. In further searching for interaction between FMRF-NH2 and morphine, it was found that 1) morphine, infused through the subarachnoidal space of spinal cords, induced FMRF-NH2-IR release and 2) opiate receptor of rat brain as measured by radioactive dihydromorphine was altered by FMRF-NH2. The results taken together suggest an important role for FMRF-NH2-IR in opiate antinociception. The role of FMRF-NH2-IR in chronic morphine tolerance will be further investigated.

Project Description:

We have previously observed that both CCK-8-SO₄ and FMRF-NH₂ injected intrathecally attenuated the antinociception induced by morphine. FMRF-NH₂ is a neuropeptide of clam origin, however, presence of FMRF-NH₂ like immunoreactive material (FMRF-NH₂-IR) in mammalian CNS has been well established.

In this study, FMRF-NH₂ antibody and proglumide were used as research tools to assess the roles of endogenous FMRF-NH₂-IR and CCK-8-SO₄ as modifiers of opiate antinociception. Rats were injected subcutaneously with morphine repeatedly at 2 hour intervals and their tail flick latencies after exposure to radiant heat were measured. The antinociceptive responses to morphine treatments decreased gradually and nearly complete tolerance was observed after the 7th or 8th injection. If IgG from FMRF-NH₂ antiserum was injected intrathecally at 4 hour intervals, potentiation of morphine analgesia was observed throughout the course of morphine treatments. The IgG from control serum failed to show such an effect. Proglumide, when given concurrently with the opiate, also potentiated the morphine induced antinociception. Interestingly, we found that the antimorphine activity of FMRF-NH₂ as well as that of CCK-8-SO₄ were partially reversed by proglumide. This result raises the possibility that a modification of the antagonism of opiate action by endogenous FMRF-NH₂-IR may contribute to the potentiating effect of proglumide on morphine antinociception. To probe the validity of such an assumption, it was investigated whether morphine releases FMRF-NH₂ and whether FMRF-NH₂ changes the characteristics of opiate receptor. Subarachnoidal spaces of rat spinal cord was perfused with artificial CSF using push and pull cannulae as described by Yaksh et al. (*Brain Res.* 242: 279-290, 1982). When 10⁻⁶M morphine was included in the perfusion medium, release of FMRF-NH₂-IR into subarachnoidal space was observed. Using ³H-dihydromorphine as opiate receptor ligand, it was found that FMRF-NH₂ decreased B_{max} and increased K_d for the high affinity site of brain opiate receptor. The results taken together suggest that prolonged occupancy of opiate receptor may lead to the release of FMRF-NH₂-IR to attenuate the number of opiate receptors occupied by morphine.

This study reveals an interaction between persistent opiate receptor occupancy and release of FMRF-NH₂-IR, this interaction proposes a new model to study opiate tolerance.

The proposed course of this study is to explore the role of FMRF-NH₂-IR in chronic morphine tolerance.

Publications:

Tang, J., Chou, J., Iadarola, M., Yang, H.-Y.T., and Costa, E.: Proglumide prevents and reduces acute morphine tolerance. Neuropharmacology, in press.

Tang, J., Yang, H.-Y.T., and Costa, E.: Inhibition of spontaneous and opiate modified nociception by an endogenous neuropeptides with FMRF-NH₂-like immunoreactivity. Proc. Natl. Acad. Sci. USA, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
 NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01570-02 SMRP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Synthesis of the Neurotransmitter Pool of Glutamate in the Brain

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	J.T. Wroblewski	Visiting Fellow	SMRP	NIMH
Others:	W.D. Blaker	Staff Fellow	SMRP	NIMH
	J.L. Meek	Pharmacologist	SMRP	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

High Pressure Liquid Chromatography Group

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MAN-YEARS

1.0

PROFESSIONAL:

1.0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Although glutamate is a major excitatory neurotransmitter in the brain, the specific route of its synthesis is unknown. A possible precursor is ornithine, which is known to be converted in vitro to glutamate via the enzyme ornithine aminotransferase (OAT). We previously found that inhibition of this enzyme in the septum in vivo led to the loss of glutamate, the kinetics of which was sensitive to an acute lesion of the glutamatergic afferents. In this study, tracer techniques were used to determine if the conversion of ornithine to glutamate proceeds in vivo and if canaline blocks such conversion. Intraventricular administration of 3H-ornithine led to the labeling of septal glutamate. This labeling was largely blocked by intraseptal canaline, but the conversion of 14C- α -ketoglutarate to glutamate was unaffected. Thus, canaline specifically blocks the formation of glutamate from ornithine. This finding, in connection with the previous lesion studies, supports the role of ornithine as a precursor for the neurotransmitter pool of glutamate.

Project Description:

Although glutamate is a major excitatory neurotransmitter in the CNS, the identity of the precursor(s) of the neurotransmitter pool of glutamate, as opposed to the other metabolic pools of glutamate, is unknown. It has been shown that the in vivo inhibition of the enzyme ornithine aminotransferase (OAT) by the intraseptal injection of canaline leads to the loss of a small pool of septal glutamate with a half life of 7 minutes. Since the kinetics of this loss is sensitive to an acute lesion of the glutamatergic afferents to the septum (i.e., fimbrial lesion), it seems likely that OAT may be involved in the maintenance of the neurotransmitter pool of glutamate.

The objective of the present study was to use tracer techniques to determine if the reaction catalyzed by OAT (the conversion of ornithine and α -ketoglutarate to glutamate and γ -glutamic semialdehyde) takes place in vivo and if canaline injection indeed blocks the reaction.

The methodology used consisted of an automated HPLC apparatus for the separation and quantitation of amino acids and the determination of label incorporation into them after the intraventricular injection of ^3H -ornithine and ^{14}C - α -ketoglutarate. The effect of canaline on such incorporation was determined by the intraseptal injection of canaline 5 minutes prior to the administration of the labeled compounds.

Major Findings:

- 1) Injection of ^3H -ornithine led to the labeling of septal glutamate, glutamine, GABA and proline. ^{14}C - α -Ketoglutarate labeled glutamate, glutamine, and GABA, but not proline. The labeling patterns and time courses are consistent with those expected based on the known substrates and products of OAT and demonstrate the existence in the brain of a pathway converting ornithine to glutamate and associated amino acids.
- 2) Intraseptal canaline reduced the label incorporation from ^3H -ornithine by 6-9 fold, but did not affect that from ^{14}C - α -ketoglutarate. This indicates that canaline blocked the conversion of ornithine to glutamate, which can occur only through OAT, but had no effect on the conversion of α -ketoglutarate to glutamate, which can occur by a variety of routes.

Proposed Course:

- 1) Canaline appears to both affect a neurotransmitter pool of glutamate and specifically block the conversion of ornithine to glutamate. Brain slice and push-pull cannula techniques will be employed to see if ornithine preferentially labels a pool of glutamate which is releasable in a Ca^{2+} dependent manner (i.e., neurotransmitter pool).
- 2) Further tests of the specific labeling of neurotransmitter glutamate by ornithine will be made by analyzing the effect of fimbrial lesions on the in vivo conversion of ^3H -ornithine to glutamate in the septum.
- 3) If these studies are positive, a turnover method for neurotransmitter glutamate will be pursued using ^3H -ornithine as a precursor.

Significance:

There are numerous indications that glutamate as a major excitatory transmitter may be involved in several degenerative neurological disorders such as Huntington's chorea, Parkinsons disease and spinocerebellar ataxia, as well as in the mechanisms of epilepsy and post-ischemic brain damage. Suggestions exist concerning the role of cortical glutamatergic efferents in altering the control of cholinergic neurons in Alzheimer's disease. The possibility to discriminate between the transmitter and metabolic pools of glutamate, and to study the activity and pharmacology of central glutamatergic pathway may contribute to a better understanding of the pathology of the above disorders.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 MH 01571-02 SMRP

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
GABA/Benzodiazepine Receptor Complex in Adrenal Medulla

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Y. Kataoka	Visiting Fellow	SMRP	NIMH
Others:	Y. Gutman	Visiting Scientist	SMRP	NIMH
	A. Guidotti	Section Chief	SMRP	NIMH
	E. Costa	Lab Chief	SMRP	NIMH
	I. Hanbauer	Pharmacologist	HE	NHBLI

COOPERATING UNITS (if any)

Natl. Heart, Lung, Blood Inst., Div. Hypertension-Endocrine Branch, Bethesda, Md.

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Neuroendocrinology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MAN-YEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0
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CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Adrenal chromaffin cells contain high affinity saturable benzodiazepine and GABA binding sites. The pharmacological specificity and the interaction between these two binding sites is similar to that reported in brain. However, the benzodiazepine binding sites of adrenal medulla cells have a low affinity (100 nM) for 3-carboxylate esters of beta-carbolines. Adrenal chromaffin cells also contain γ -aminobutyric acid, glutamic acid decarboxylase, GABA aminotransferase, and GABA receptors appear to modulate the acetylcholine-induced release of catecholamines and enkephalin-like peptides in opposite directions.

Project Description:

It has been reported that the behavioral effects of anxiolytic benzodiazepines or anxiogenic beta-carbolines are associated with concomitant peripheral effects due to central stimulation of adrenal medullary function. To elucidate whether these peripheral effects are the consequence of a direct action of benzodiazepine ligands on the adrenal medullary cells, we have examined whether adrenal chromaffin cells contain specific recognition sites for benzodiazepines and GABA. Binding studies using ^3H -flunitrazepam indicate that this ligand binds to membranes obtained from cow adrenal medulla or from primary cultured cells with a K_d of approximately 10 nM and a B_{max} of approximately 80 fmol/mg prot. The binding of ^3H -flunitrazepam is displaced (more than 50%) by diazepam, RO 15-1788 and beta-carboline methyl ester. However, diazepam is 10-20 fold more potent than RO 15-1788 or beta-carboline methyl ester (IC_{50} 100 nM). The low affinity of beta-carboline methyl ester for the benzodiazepine binding sites in adrenal medulla was confirmed by direct binding measurement using ^3H -beta-carboline methyl ester. ^3H -RO 4864, the ligand for the peripheral benzodiazepine binding sites, failed to bind specifically to the adrenal medulla membranes in the concentration range from 0.4 to 200 nM. ^3H -Flunitrazepam binding was increased by 30-40% by the addition of 100 μM GABA to the incubation medium. This increase was similar to that observed in brain.

The chromaffin cells of adrenal medulla also contain γ -aminobutyric acid (GABA), glutamic acid decarboxylase, GABA aminotransferase and GABA receptors. Stimulation of these receptors down-regulates the acetylcholine-induced release of catecholamine (CA) in primary cultures of chromaffin cells. In these cultures 1 μM of bicuculline (GABA antagonist) facilitates the release of CA caused by nicotinic receptor stimulation but fails to change the release of CA evoked by KCl depolarization.

Since opioid peptides coexist with CA in the vesicles of chromaffin cells and nicotinic receptor stimulation releases both neuromodulators, we have investigated whether bicuculline changes the release of met-enkephalin like peptide elicited by nicotine. Bicuculline (1 μM) inhibited the release of met-enkephalin by various doses of nicotine without changing the spontaneous release of met-enkephalin.

To evaluate the physiological role of GABA in medullary function in vivo, the release of CA from adrenal medulla was studied in anesthetized (pentobarbital 0.43 $\mu\text{g}/\text{kg}$ i.v.) American Foxhound dogs weighing 20-25 kg. After the transection of splanchnic nerve, blood was collected from the lumbar adrenal vein and drugs were injected through the cannula placed on the femoral vein. Plasma CA was isolated by Al_2O_3 adsorption and measured by HPLC coupled to an electrochemical detector. THIP (GABA agonist) (5 mg/kg) decreased the release of CA evoked by splanchnic stimulation (10 V/3-6 Hz), while bicuculline (0.5-1.0 mg/kg) increased it. The effects caused by THIP and bicuculline were more pronounced on the release of epinephrine than in that of norepinephrine. These results support the view that intrinsic GABAergic mechanisms may modulate chromaffin cells responsiveness to incoming cholinergic stimuli.

The present observations provide the first demonstration for a benzodiazepine GABA receptor complex of central type outside of the CNS. Moreover this study

unequivocally provides evidence that this GABA-benzodiazepine receptor complex has an affinity for the 3-carboxylic acid esters of beta-carboline much lower than that of brain.

Publication:

Kataoka, Y., Gutman, Y., Guidotti, A., Panula, P., Wroblewski, Y., Cosenza-Murphy, D., Wu, J.Y., and Costa, E.: The intrinsic GABAergic system of adrenal chromaffin cells. Proc. Natl. Acad. Sci. USA 81: 3218-3222, 1984.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 01572-02 SMRP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Endogenous Effector for Benzodiazepines

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	P. Ferrero	Guest Researcher	SMRP	NIMH
Others:	D. Konkel	Chemist	SMRP	NIMH
	H. Alho	Visiting Associate	SMRP	NIMH
	D. Cosenza-Murphy	Biologist	SMRP	NIMH
	A. Guidotti	Section Chief	SMRP	NIMH
	E. Costa	Lab Chief	SMRP	NIMH

COOPERATING UNITS (if any)

B. Conti-Tronconi, Division of Chemistry and Chemical Engineering,
California Inst. Technol., Pasadena, CA 91125

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Neuroendocrinology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MAN-YEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The presence in synaptic membranes of high affinity recognition sites for benzodiazepines capable of eliciting biochemical, physiological or behavioral responses when acted upon by specific ligands, prompted the search for an endogenous ligand that operates in physiological conditions. The existence of an endogenous modulator for the benzodiazepine-GABA receptor complex was suggested by an early observation that the KD for 3H-diazepam binding to GABA-free crude synaptic membranes was reduced by approximately 50% by repeated washes of the membranes with small concentrations of detergents. It was soon discovered that the membrane extract contained a peptide which produced a dose-related increase of KD of diazepam binding without affecting the Bmax. Recently we developed a relatively simple procedure to isolate and purify this peptide to homogeneity. The peptide was termed DBI (diazepam binding inhibitor) and its extraction was routinely carried out using as a starting material rat brain homogenized in hot (80°) 1 N acetic acid. Purification was achieved using Sephadex G-100 and G-75 chromatography and reverse phase HPLC. Studies with the antibody reveal that DBI is unevenly distributed in brain and behavioral studies indicate that DBI acts like beta-carboline in facilitating punished behavior.

Project Description:

DBI (diazepam binding inhibitor) extracted from rats or humans was purified to homogeneity as indicated by the presence of a single band of protein on SDS, on acidic urea gel electrophoresis, and on three different columns and solvent systems for HPLC. It contains 104 amino acids with an abundance of lysine residues, is basic in nature, and has a MW of approximately 11,000 daltons. In addition, the HPLC peak with DBI activity contains tyrosine as a single carboxy-terminus.

Experiments to establish the amino acid sequence of this purified peptide revealed that the N-terminal amino acid is blocked. This block could not be resolved by several attempts carried out until now. The presence of 2-methionine in the molecule, allowed for the generation of 3 fragments following cyanogen bromide treatment. The sequence of the carboxy-terminal fragment was determined, and a partial sequence of the middle fragment was determined. These sequences do not resemble any known mammalian peptide sequence. The fragment containing tyrosine as its carboxy-terminus probably represents the terminal fragment of the molecule.

When DBI extracted from rat or human brains is injected i.c.v. into thirsty rats subjected to the Vogel test, it fails to have any anticonflict action, but in contrast, lowers the threshold for the suppression of punished behavior and blocks the anticonflict action of diazepam. The action of DBI in this behavioral test is indistinguishable from that of beta-carboline because similarly to beta-carboline the effect of DBI is blocked by RO 15-1788 and is potentiated by a decrease of GABAergic transmission induced by isoniazid. These data suggest that DBI may act as a naturally occurring anxiogenic compound regulating GABAergic transmission. However, the question of whether DBI represents the physiologically relevant endogenous cotransmitter operative in GABAergic transmission remains unanswered by these experiments. To answer this question, we began several groups of experiments to establish DBI distribution in brain, its synaptic and cell localization, its coexistence with GABA/benzodiazepine receptor system, and its action on the chloride channel that is regulated by GABA. Using an antiserum prepared in rabbit by injecting purified DBI with Freund's adjuvant we could determine DBI-like immunoreactivity in various brain structures. An initial survey indicates that cerebellum is among the brain areas with the highest content of DBI-like immunoreactivity, followed by pons medulla, hippocampus, striatum and cortex. Histochemical studies indicate particularly high concentrations of DBI-like immunoreactivity in the molecular layer of cerebellum and the outer layers of cortex. Since these areas of brain are particularly rich in benzodiazepine and GABA receptors, this type of anatomical relation between DBI and GABAergic system is in line with the idea that DBI may play some role in the control of GABA receptor function. The large molecular weight of DBI and its relatively weak potency (K_i in the μM range) as an inhibitor of benzodiazepine binding suggest we are isolating a precursor of a smaller molecular weight active peptide. This smaller peptide would be the natural endocoid of benzodiazepine receptor that acts as an agonist on this recognition site.

The part of DBI that was sequenced shows the presence of 2-methionine and many pairs of basic amino acid residues flanking a small chain of polypeptides. We took advantage of these characteristics to cleave DBI in smaller peptide fragments using cyanogen bromide (CNBr) and trypsin. CNBr produced three major fragments. CNBr-2

and CNBr-3 fragments which represent the middle and carboxy-terminal fragment of DBI, have a molecular weight of 3200 and 1800, respectively, and are ineffective in displacing ^3H -diazepam from its binding sites at the synaptic membrane level or in producing a proconflict action when injected intraventricularly into rats. CNBr-1 fragment, which includes the amino-terminal portion of DBI has a molecular weight of approximately 6000 daltons, displaces ^3H -diazepam binding, and elicits proconflict action when injected in rat. Trypsin digestion produces 10 major peptide fragments. Of these fragments only fragment T6 (MW of approximately 2000 daltons) has proconflict action. These data suggest that DBI may function as a precursor of a putative endocoid of the benzodiazepine recognition site.

The fact that DBI behaves like a beta-carboline derivative raises the possibility that it may not be the perfect endogenous ligand of benzodiazepine recognition site, because it may mimic beta-carboline derivatives rather than benzodiazepines. If this is the only endogenous ligand for the benzodiazepine recognition site in rat brain then we must say that brain possesses only an endogenous anxiety mechanism and that it is an anxiogenic rather than anxiolytic mechanism. However the possibility that there are two sets of endogenous peptides, one mimicking the benzodiazepines and the other mimicking the beta-carbolines, cannot be excluded at this time.

Benzodiazepines are widely used to treat patients with pathological anxiety. Now new research suggests that benzodiazepines correct an imbalance in the GABA benzodiazepine receptor system. This imbalance may be linked to naturally-occurring chemicals that work through the same brain cell mechanisms as benzodiazepines. Our work has uncovered what appears to be a natural substance that induces anxiety. If this observation is confirmed, we believe purification of such substance would revolutionize the treatment of anxiety.

Publications:

Costa, E., Corda, M.G., and Guidotti, A.: On a brain polypeptide functioning as a putative effector for the recognition sites of benzodiazepine and beta carboline derivatives. Neuropharmacology 22: 1481-1492, 1983.

Guidotti, A., Corda, M.G., Vaccarino, F.M., and Wise, B.C.: Role of GABA-modulin and of an endogenous effector of beta-carboline binding sites in the GABA-benzodiazepine receptor interaction. In Bowery, N. (Ed.): Action and Interactions of GABA and Benzodiazepine. New York, Raven Press, 1984, pp. 191-202.

Corda, M.G., Ferrari, M., Guidotti, A., Konkell, D., and Costa, E.: Isolation, purification and partial sequence of a neuropeptide (diazepam-binding inhibitor) precursor of an anxiogenic putative ligand for benzodiazepine recognition site. Neurosci. Lett., in press.

Costa, E., Ferrari, M., Ferrero, P., and Guidotti, A.: Multiple signals in GABA-ergic transmission: Pharmacological consequences. Neuropharmacology, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01573-02 SMRP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

A Search for Models to Study Receptors "In Vivo" by Emission Computed Tomography

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	P. Ferrero	Guest Researcher	SMRP	NIMH
Others:	A. Guidotti	Section Chief	SMRP	NIMH
	E. Costa	Lab Chief	SMRP	NIMH

COOPERATING UNITS (if any)

G. Di Chiro, NINCDS, Neuroradiology and Emission Computed Tomography Section, Bethesda, Md.

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Neuroendocrinology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MAN-YEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

High specific radioactivity ligands for neurotransmitter receptors can be used to characterize in vivo the functional status of the receptor. Ideally this should be done with non-invasive brain imaging techniques, such as emission computed tomography. While resolution of the apparatus improves the study of receptors in several brain nuclei we have decided to prepare appropriate models for these studies. With an ideal ligand, one can study regional distribution, saturability and pharmacological specificity of the binding sites and characterize the various functional states of the receptor. To study DA receptors *ex vivo*, we have selected 3H-spiroperidol. This drug is almost an ideal probe for binding studies in vivo because it is poorly metabolized in brain and binds with high affinity to the DA receptors. The data obtained in animal models could be extended to measure DA receptor function in man by injecting spiroperidol labeled appropriately for ECT scanning. To study the GABA receptor, we used 3H-muscimol. Muscimol binds with high affinity and in a saturable manner to GABA-A receptors. Diazepam, 1 mg/kg, increases the Bmax of this binding but fails to change the affinity of 3H-muscimol to different brain areas. In this study we could demonstrate a good correlation between receptor occupancy and behavioral effect of GABA-mimetic.

Project Description:

The content of authentic ^3H -spiroperidol and of its metabolites was measured in brain regions of rat, guinea pig and mouse receiving tracer doses of ^3H -spiroperidol intravenously (0.2 to 0.5 $\mu\text{g}/\text{kg}$). The change of the ^3H -spiroperidol content with time in various brain regions shows that a steady state was maintained between 2 and 6 hrs post injection; the lowest ^3H -spiroperidol content was attained in cerebellum where the value approached that of blood plasma. Since the cerebellum contains an insignificant number of dopamine receptors but many serotonin receptors and other sites that bind ^3H -spiroperidol, the ^3H -spiroperidol contained in cerebellum was considered background binding. In the striatum and olfactory tubercle of rats receiving for 3 weeks two daily doses of haloperidol or amphetamine, the amount of ^3H -spiroperidol that binds in vivo is either decreased or increased, respectively. If the kinetic characteristics of in vivo binding of ^3H -spiroperidol observed in the rat, guinea pig and mouse can be replicated in man using spiroperidol containing a γ - or α positron-emitting label, one might have a probe to study the dynamics of dopamine receptors in vivo in various brain nuclei with emission computed tomography scanning. One could determine up- and down-regulation of receptors and express the dynamic state of the receptor.

In vitro measurements of affinity constants for binding of dopamine (DA) ligands to crude synaptic membranes are very useful to characterize DA receptor recognition sites and to describe their density in various brain areas. For instance, when occupancy of DA recognition sites is decreased because of a lesion of nigrostriatal dopaminergic neurons or following chronic treatment with neuroleptic drugs, the number of ^3H -neuroleptic binding sites is increased. Moreover, binding studies in postmortem material have shown that schizophrenia and Parkinson's disease affect ligand binding to DA recognition sites. Since contrasting opinions exist on the validity of extrapolating postmortem binding studies to the in vivo situation, it is hoped that emission computed tomography (ECT) scanning, in its two modalities, that with γ -emitters (single photon emission tomography (SPECT) and that with positron emitters (PET) may be used to locate and diagnose DA receptor abnormalities in vivo in man.

Before undertaking human studies, it is necessary to evaluate and adapt animal models to detect the in vivo modulation of receptor number or affinity that may occur in various pathological conditions. These models may help to define whether sub- and supersensitivity of receptors occur in human pathology.

If it could be demonstrated that the ^3H -spiroperidol kinetic found in the three species applies also to man, spiroperidol appropriately labeled could be used advantageously for ECT scanning. Considering that the steady state is reached at 2 hrs, a long living radiolabel (^{18}F as opposed to ^{11}C) would be preferable for PET studies. ECT scanning measurement could be carried out in the same individual at different intervals in order to establish a base line, and the administration of subpharmacological amounts of neuroleptics could be used to obtain a displacement curve to study the relative abundance, and the kinetic characteristics of DA_1 and DA_2 receptors.

Using the experience acquired with spiroperidol, we extended this study to other receptor ligands. In particular, we initiated studies on the in vivo binding of ^3H -ligand to the GABA recognition sites.

γ -Aminobutyric acid (GABA) receptors were characterized in vivo by studying ex vivo the binding of ^3H -muscimol to cerebellum, cortex, hippocampus, and corpus striatum of mice receiving intravenous injections of tracer doses of high-specific-activity (≈ 30 Ci/mmol) ^3H -muscimol. This ligand binds with high affinity (apparent K_d , $2-3 \times 10^{-9}\text{M}$) to a single population of binding sites (apparent B_{max} , 250-180 fmol per 10 mg of protein). Pharmacological studies using drugs that selectively bind to GABA_A or GABA_B receptors suggest that ^3H -muscimol specifically labels a GABA_A recognition site. Moreover, diazepam (1.5 $\mu\text{mol/kg}$, i.p.) increases the B_{max} but fails to change the affinity of ^3H -muscimol binding to different brain areas. This diazepam-elicited increase in B_{max} is blocked in mice receiving the diazepam agonist RO 15-1788 (ethyl-8-fluoro-5,6-dihydro-5-methyl-6-oxo-4H-imidazo [1,5a] π [1,4] benzodiazepine-3-carboxylate). Since the diazepam-induced increase of ^3H -muscimol binding is parallel to a significant potentiation of the inhibitory effect of muscimol on locomotor activity, it is proposed that the facilitatory action on GABAergic transmission elicited in vivo by diazepam is mediated by an increase in the B_{max} of the binding sites of GABA_A receptors.

We intend to: 1) continue the characterization of D_1 and D_2 receptor in vivo using specific ligands of D_1 and D_2 recognition sites and 2) explore the interaction of muscimol receptors with different benzodiazepines and beta-carbolines.

Publications:

Ferrero, P., Vaccarino, F., Guidotti, A., Costa, E., and Di Chiro, G.: In vivo modulation of brain dopamine recognition sites: A possible model for emission computed tomography studies. Neuropharmacology 22: 791-795, 1983.

Ferrero, P., Guidotti, A., and Costa, E.: Increase in the B_{max} of γ -aminobutyric acid: A recognition site in brain regions of mice receiving diazepam. Proc. Natl. Acad. Sci. USA 81: 2247-2251, 1984.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 01574-02 SMRP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Peptide Ligands for Nicotinic Receptors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: K. Kageyama Guest Researcher SMRP NIMH

Other: A. Guidotti Section Chief SMRP NIMH

COOPERATING UNITS (if any)

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TOTAL MAN-YEARS:

0.6

PROFESSIONAL:

0.6

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

In primary cultures of bovine chromaffin cells, commercially available preparations of α -bungarotoxin inhibit the acetylcholine (ACh)- or nicotine-evoked release of endogenous catecholamines. The potency of different lots of α -bungarotoxin is not related to the α -bungarotoxin peptide content but to that of another peptide (termed P-4 bungarotoxin) present as an impurity in the α -bungarotoxin preparations. P-4 Bungarotoxin was isolated and purified to homogeneity by high-pressure liquid chromatography (HPLC). Homogeneity was established by a variety of means, including polyacrylamide gel electrophoresis, HPLC, end carboxy group analysis and NH₂-terminal amino acid sequence. Purified P-4 bungarotoxin contains approximately 121 amino acid residues, and it is different in its amino composition, molecular weight, and amino acid sequence from α -bungarotoxin and β -bungarotoxin. P-4 Bungarotoxin (IC₅₀ \approx 1 nM) blocked the ACh-induced release of endogenous catecholamines but failed to block the KCl-induced catecholamine release. Although P-4 bungarotoxin is endowed with phospholipase A₂ activity, its effect on ACh-evoked catecholamine release persists when the phospholipase activity is blocked (99.9%) by treatment of the toxin with p-bromophenacyl bromide. P-4 Bungarotoxin may represent a useful tool with which to study nicotinic receptor function in sympathetic and central nervous system neurons.

Project Description:

Using as starting material commercially available preparations of α -bungarotoxin or native *Bungarus multicinctus* venom, a 15,000 Mr toxin has been purified to homogeneity. This toxin has been partially sequenced and termed P-4 bungarotoxin. P-4 Bungarotoxin in the nanomolar concentration range acts as an apparent competitive inhibitor of nicotinic receptor stimulation of primary cultures of cow adrenal chromaffin cells.

The P-4 toxin is present in a small percentage (1-10%) in both native snake venom and α -bungarotoxin preparations. Thus, it could be suggested that P-4 bungarotoxin is a precursor of α -bungarotoxin. However, by comparing the amino acid composition of P-4 bungarotoxin and α -bungarotoxin and the amino acid sequence of the two toxins, it is evident that the α -bungarotoxin peptide is not contained in the P-4 molecule. The NH₂-terminal sequence of the first 10 residues of P-4 bungarotoxin (Asn-Leu-Tyr-Gln-Phe-Lys-Asn-Met-Ile) is highly homologous with that of various snake venom toxins endowed with phospholipase activity. Moreover, the sequence of P-4 bungarotoxin from the amino acid residues 1-55 is identical with the sequence of phospholipase A from *Bungarus multicinctus* venom and shows a high degree of homology with the A chain of β_1 -bungarotoxin. Although P-4 bungarotoxin, like β -bungarotoxin, possesses phospholipase activity, it is apparently different from the β -bungarotoxins because it has a smaller molecular weight and a different amino acid composition.

P-4 Bungarotoxin is apparently different from Berger bungarotoxin 3.1 and 3.3 because it has a larger molecular weight, but it could have some similarities to the Quick and Lamarca 15,000 Mr peptide. P-4 Bungarotoxin in the nanomolar concentration range not only blocks the acetylcholine-induced catecholamine release in adrenal cells (its effect is slowly reversible) but, similar to Quick and Lamarca peptide, also blocks ganglionic transmission in frog sympathetic ganglia reversibly.

A possible mode of action of P-4 bungarotoxin could be linked to the phospholipase activity that we have found to be associated with the peptide. It has been postulated, for example, that β -bungarotoxin, which also possesses phospholipase activity, reduces cholinergic transmission at least in part by decreasing the amount of ACh released from presynaptic nerve terminals. This effect of β -bungarotoxin can be prevented when the phospholipase activity is blocked by treating the toxin with p-bromophenacyl bromide. In the case of P-4 bungarotoxin, however, we can show that inhibition of phospholipase activity (more than 99.9%) does not abate the P-4 bungarotoxin inhibition of ACh-induced catecholamine release. Therefore, the results suggest that P-4 bungarotoxin may be acting by blocking ACh-nicotinic receptors in cultures of bovine chromaffin cells.

This research may lead to the identification of a ligand for the nicotinic receptors of mammalian CNS. Since the function of nicotinic receptors in CNS has never been carefully investigated, the approach we have taken can be of great importance in understanding the role of this receptor in the control of brain function. If we are successful in labeling this ligand we may have an interesting tool to study in detail the role of nicotinic receptor in Alzheimer disease.

We propose:

- 1) To develop a method to obtain high specific activity labeled P-4 bungarotoxin without loss of its biological activity.
- 2) Explore the behavioral and biochemical effect of P-4 bungarotoxin injected directly into the lateral ventricles in experimental animals.

Publications:

Kageyama, H., and Guidotti, A.: Effect of modulators of nicotinic receptor function on endogenous and radiolabelled catecholamine release from primary cultures of adrenal chromaffin cells. J. Neurosci. Meth. 10: 9-16, 1984.

Saiani, L., Kageyama, H., and Guidotti, A.: Purification and characterization of a bungarotoxin polypeptide which blocks nicotinic receptor function in primary culture of adrenal chromaffin cells. Mol. Pharmacol. 25: 327-334, 1984.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 01575-01 SMRP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Genetically Epilepsy-Prone Rat

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	H. Laird II	IPA Appointee	SMRP	NIMH
Others:	M. Hadjicostantinou	Guest Researcher	SMRP	NIMH
	N.H. Neff	Section Chief	SMRP	NIMH

COOPERATING UNITS (if any)

None

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NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MAN-YEARS:

0.4

PROFESSIONAL:

0.4

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is to study the neurobiochemistry of the genetically epilepsy-prone rat. Our immediate objective is to evaluate the central cholinergic neurons in various regions of the brain of these animals.

Project Description:

The genetically epilepsy-prone rat (GEPR) is a useful model for studies of the epilepsies. These animals exhibit a generalized predisposition to seizures. They have a lower threshold and more intense seizure for a given stimulus, electrical or chemical, than other strains of rats. Moreover, the adult GEPR is susceptible to hyperthermic and audiogenic seizures, whereas control rats are not. Thus, like the human, the GEPR has a genetically determined decreased ability to suppress seizures once the process has been initiated. After treatment with drugs that alter the brain content of biogenic amines, the ratio of catecholamines and/or indoleamines to acetylcholine in brain is generally a measure of whether a test animal will be susceptible to seizure-inducing procedures. The higher the ratio the more resistant the animals. Brain content of catecholamines and indoleamines are known to be low in GEPRs. Our objective is to evaluate cholinergic neurons in the brain of normal animals and GEPRs.

Acetylcholine and choline were analyzed in the brain of control rats and GEPRs by HPLC.

A breeding colony of GEPRs has been established by our laboratory. Our preliminary studies of cholinergic neurons in GEPR has revealed a significantly higher content of choline and acetylcholine in several, but not all regions of brain. For some regions there is about a 100 percent increase over control values. These regions include frontal and parietal cortex, thalamus, midbrain and striatum. Indeed, these are the regions of brain where the catecholamines and indoleamines are lower than normal. These findings support the notion derived from normal animals treated with drugs that there is a dynamic balance between the biogenic amines and acetylcholine for the modulation of seizure activity.

The GEPR is one of the few models of a genetically related brain dysfunction. These animals present a unique opportunity for studying the molecular mechanisms of a genetic brain disorder. A genetic component has been identified with some forms of mental disease. The GEPR may serve as a model for understanding these disorders.

The research will be terminated and the work prepared for publication.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 01576-01 SMRP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Neuropharmacology of N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	M. Hadjiconstantinou	Guest Researcher	SMRP	NIMH
Others:	H. Laird II	IPA Appointee	SMRP	NIMH
	E. Anthopoulos	Guest Researcher	SMRP	NIMH
	N.H. Neff	Section Chief	SMRP	NIMH

COOPERATING UNITS (if any)

None

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TOTAL MAN-YEARS:

0.5

PROFESSIONAL:

0.5

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

MPTP (N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) is a side product of meperidine synthesis. When given to man and primates it induces persistent symptoms of parkinsonism and the clinical signs can be reversed by administering L-DOPA or bromocriptine. In rhesus monkey, MPTP induces degeneration of the nigrostriatal pathway with a concomitant reduction of dopamine. Our objective was to determine if similar degenerative changes of dopaminergic neurons could be induced in mice and to determine if the acetylcholine content of cholinergic neurons might be altered by the loss of dopaminergic neurons.

Project Description:

Clinical signs similar to parkinsonism develop in man after the intravenous self-administration of N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). MPTP is a side product in the synthesis of meperidine and it may be found in some illicit meperidine preparations. The rhesus monkey also develops symptoms similar to parkinsonism and there is degeneration of the nigrostriatal pathway with loss of dopamine. The clinical signs can be reversed by administering L-DOPA or bromocriptine. Our objective was to determine if similar degenerative changes of dopaminergic neurons might be induced in mice and to determine if cholinergic neurons might be affected as they are thought by many investigators to be innervated by dopaminergic neurons.

MPTP was administered to mice intravenously and catecholes, choline and acetylcholine assayed by HPLC.

Mice were given two 10 mg/kg doses, 16 hours apart, of MPTP and killed at selected times over eight days after the second dose. Most regions of the CNS including, striatum, frontal cortex, spinal cord and retina contained significantly less dopamine and its metabolite DOPAC 8 days later. The depletion was not specific for dopaminergic neurons as norepinephrine was depleted as well. In contrast to the catecholamines, both choline and acetylcholine were generally elevated, in some regions by as much as 100 percent, after MPTP.

Our studies demonstrated that mice can be used to study the neurotoxicology of MPTP. Moreover, our studies indicate that the drug is not selective, both noradrenergic and dopaminergic neurons appear to be affected. The loss of catecholamines appears to parallel the rise of acetylcholine in brain. The importance of MPTP is not that it induces long-term depletion of the catecholamines, but that it may provide a clue as to why the catecholamine neurons are more vulnerable than other neurons. Parkinsonism usually leads to dementia thus MPTP may provide valuable new information on dementia as well.

The studies will be terminated and the work prepared for publication.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 01577-01 SMRP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Characterization of Serotonin Pre- and Postsynaptic Components in NCB-20 Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	T. Nakaki	Visiting Fellow	SMRP	NIMH
Others:	B.L. Roth	Guest Researcher	SMRP	NIMH
	D.M. Chuang	Chemist	SMRP	NIMH
	E. Costa	Lab Chief	SMRP	NIMH

COOPERATING UNITS (if any)

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SECTION

Monoclonal Antibody Group

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MAN-YEARS:

1.8

PROFESSIONAL:

1.8

OTHER:

0

CHECK APPROPRIATE BOX(ES).

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Since serotonin (5-HT) synapse has been implicated to be an important site of action of antidepressant drugs, we have searched for a cultured cell system that can be used as a model to study the interactions between recognition sites for antidepressant drugs and receptors for 5-HT. We have found that 5-HT sensitive adenylate cyclase located in NCB-20, a hybrid cell line, can be effectively blocked by ketanserin (with a $K_i = 50 \text{ nM}$) which is a selective antagonist for 5-HT₂ receptors. The membrane preparation of NCB-20 cells also possesses high affinity binding sites for ketanserin, and mianserin, an atypical antidepressant. The binding parameters for ketanserin and mianserin are comparable to those reported in the membrane preparation of rat brain. In addition this cell line is equipped with a serotonin uptake system which is partially sodium dependent and is inhibited by fluoxetine, a specific blocker of 5-HT uptake and imipramine and desipramine, both are typical tricyclic antidepressants. The high affinity binding site for imipramine in this cell line has a B_{max} (about 16 pmol/mg protein) which is at least 50 times higher than reported in the rat brain. Thus NCB-20 cell line is a convenient model to study the interactions between antidepressant drugs and the recognition sites for the various chemical signals participating in the transaction of communication of 5-HT synapses. This study should lead to a better knowledge of the molecular mechanisms that follows the binding of antidepressants to specific recognition sites in the target tissue.

Project Description:

Serotonin (5-hydroxytryptamine, 5-HT) synapse has been implicated to be an important site of action for antidepressant drugs. Imipramine, a typical antidepressant, is bound to a site located in the serotonin nerve endings, this site could be a recognition site for an endocoid controlling the uptake of 5-HT in a negative manner. In contrast, mianserin, an atypical antidepressant, binds to a high affinity, postsynaptic recognition site located in the 5-HT synapse; this site is related but not identical to the 5-HT₂ receptor recognition site. Studies performed in this laboratory have provided evidence that these binding sites for antidepressants are related to the expression of this pharmacological profile such as the down-regulation of brain β -adrenergic receptors induced by daily injections of imipramine or mianserin for 2 weeks (for review see: Handbook of Neurochemistry 16: 307-330, 1984). However, it is difficult to study molecular mechanisms of drug action in brain tissues. In fact, brain has a heterogeneous cell population and the interactions between various types of neurons and glial cells are extremely complex. An ideal model system to study receptor-receptor interaction could be a cloned cell line which contains in the same membrane drug and transmitter receptors. We have investigated whether NCB-20, a cloned hybrid cell line of mouse neuroblastoma and embryonic Chinese hamster brain could be such a model system.

NCB-20 cells were shown to have 5-HT-sensitive adenylate cyclase by Nirenberg and coworkers (Proc. Natl. Acad. Sci. USA 76: 135-1139, 1979). In confirming these results, we found that the 5-HT-sensitive adenylate cyclase can be effectively blocked by ketanserin, a selective antagonist acting on the 5-HT₂ receptors. We have also found that there is a specific binding site for ³H-ketanserin in the membrane of NCB-20 cells; the values of K_d and B_{max} are 7.4±2.8 nM and 0.63±0.11 pmol/mg protein respectively. The K_i of ketanserin for the inhibition of 5-HT-sensitive adenylate cyclase is about 50 nM; ketanserin fails to inhibit the basal activity of adenylate cyclase. Specific binding site for ³H-mianserin can also be detected in NCB-20 cells with a K_d = 10.8±2.3 nM and B_{max} = 1.51±0.56 pmol/mg protein. The parameters of ³H-ketanserin and ³H-mianserin binding to NCB-20 cells are comparable to those found in the brain.

We have found that NCB-20 cells are also equipped with a 5-HT uptake system. ³H-5-HT is taken up into the cell in a saturated manner with a K_m = 7.3±0.6 μ M and V_{max} = 2.0±0.6 pmol/min/mg protein. Thin layer chromatography revealed that about 80% of the radioactivity taken up is attributed to the authentic 5-HT. The uptake is temperature-dependent and partially Na⁺ dependent, as analogous to the 5-HT uptake in neuroblastoma N2a and rat pinealocytes. The 5-HT uptake in NCB-20 is inhibited by fluoxetine, a specific inhibitor of 5-HT uptake and tricyclic antidepressants such as imipramine and desipramine. The order of potency for the inhibition is fluoxetine > imipramine > desipramine. We have also characterized the Na⁺-dependent ³H-imipramine binding site in NCB-20 cell membranes. This binding is of high affinity and saturable with a K_d = 12±2 nM and B_{max} 16±6 pmol/mg protein. It is of interest to note that the B_{max} of imipramine binding site in this cell line is at least 50 times higher than that found in the rat brain.

In conclusion, NCB-20 cell possesses presynaptic and postsynaptic components of the serotonin synapse. In addition high affinity binding sites for imipramine, mianserin and ketanserin are detected in this cell line. Thus NCB-20 cell should be an ideal model system to study the molecular events involved in the interaction

between antidepressant drugs and their receptor binding sites. The copresence of mianserin and ketanserin binding sites in this cloned cell provides us with a system to investigate the relationship and interactions between these two types of binding sites at the molecular level. The inhibition of 5-HT-dependent adenylate cyclase by ketanserin (which is in contrast to brain 5-HT-dependent adenylate cyclase) allows us to examine whether the brain polypeptide endocoid for 5-HT₂ receptor recognition sites (see other reports for details) is an agonist or antagonist to 5-HT₂ receptors. All these studies are now in progress. This investigation should lead to a better understanding of mechanism of action of antidepressants and the knowledge obtained may be used for a new basis of the therapy of affective disorders.

Publication:

Chuang, D.M., and Costa, E.: Recognition sites for antidepressant drugs. Handbook of Neurochemistry 16: 307-330, 1984.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 01578-01 SMRP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Expression of Genes for Insulin in the Brain and Peripheral Tissues

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	C.H. Lee	Chemist	SMRP	NIMH
Others:	D.M. Chuang	Chemist	SMRP	NIMH
	E. Costa	Lab Chief	SMRP	NIMH
	B.-C. Lin	Visiting Associate	LID	NIAID

COOPERATING UNITS (if any)

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TOTAL MAN-YEARS:

1.8

PROFESSIONAL:

1.8

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

This project concerns an investigation of whether or not insulin and/or insulin-like polypeptides present in brain and other extrapancreatic tissues can be de novo synthesized in situ. We have used as a probe a cloned cDNA fragment of proinsulin to assess the presence of mRNA in various tissues that can hybridize to the DNA probe. mRNA from various tissues of rat (brain and 14 peripheral tissues) and from the brain of pig, cow and human was extracted, fractionated by agarose gel electrophoresis, blotted to nitrocellulose membranes and hybridized with the radioactivity-labeled cDNA probe. We found that there are at least five mRNA species hybridizing to probe (with sizes of 560, 630, 860, 1200 and 1500 nucleotides) in the extrapancreatic tissues, while in the pancreas, only the two species with the smallest size can be found. The specific hybridization is strong in the olfactory bulb and tubercle, hypothalamus, hippocampus, brain stem, frontal cortex and cerebellum but is weak in the posterior cortex and spinal cord. Moreover, we found that mRNA from human brain shows two hybridization bands with sizes of 860 and 2000 nucleotides and mRNA from brains of pig and cow shows three hybridization bands with sizes of 1370, 2660 and 3770 nucleotides. These results may suggest that transcription of genes for insulin and/or insulin-like polypeptides occur in the brain and other extrapancreatic tissues; however, the transcriptional products are highly heterogenous. Further studies are in progress to verify the biosynthesis of these polypeptides in extrapancreatic tissues.

Project Description:

Polypeptides reactive to antibody raised against insulin have been detected in the brain and peripheral tissues of rats and humans (Havrankova et al., Nature 272: 827, 1978; Proc. Natl. Acad. Sci. USA 75: 5737, 1978; Rosenzweig et al., Proc. Natl. Acad. Sci. USA 77: 572, 1980). However it was subsequently suggested by Eng and Yalow that insulin in extrapancreatic tissues is not de novo synthesized (Diabetes 29: 105, 1980; Proc. Natl. Acad. Sci. USA 78: 4576, 1981), but is transported to these tissues through blood stream from the pancreas. In an attempt to clarify this argument and to elucidate the role of insulin and/or insulin-like materials in brain and other extrapancreatic tissues, we have used as a probe a cloned cDNA fragment of proinsulin (kindly provided by Dr. Villa-Komaroff at Univ. of Mass.) to assess the presence of mRNA species in these tissues that can be hybridized to the probe.

mRNA from various tissues of rat (brain, spinal cord, heart, lung, liver, kidney, spleen, gut, fat, testes, ovary, uterus, stomach, blood and pancreas) and brains of pig, cow and human was extracted, fractionated by agarose gel electrophoresis, blotted to nitrocellulose membrane and hybridized with the ³²P-cDNA probe which has been nick-translated with ³²P-labeled deoxyribonucleotides. The results obtained are summarized as follows: 1) There are at least five mRNA (poly A-containing messenger RNA) bands (with sizes of 560, 630, 860, 1200 and 1500 nucleotides) hybridizing to the probe for all tissues of rats. The two bands with the smallest molecular weights are found in the pancreas; the one with 630 nucleotides is the major species. In the brain, a third major band with 1500 nucleotides is also detected. 2) The specific hybridization is strong in the olfactory bulb and tubercle, hypothalamus, hippocampus, brain stem, frontal cortex, striatum and cerebellum but is weak in the posterior cortex and spinal cord. 3) The RNA samples from the olfactory bulb, brain, liver, heart and gut also show significant smearing in the hybridization pattern, suggesting the presence of other populations of mRNA species with sequence homologous to the probe. 4) mRNA from human brain shows two hybridization bands with sizes of 860 and 2000 nucleotides and 5) mRNA from brains of pig and cow shows three hybridization bands with sizes of 1370, 2660 and 3770 nucleotides. These results indicate that transcription of genes for insulin and/or insulin-like polypeptide may occur in extrapancreatic tissues and that the transcriptional products are heterogeneous in size. This suggests that there may be more than one gene coding for insulin or insulin-like polypeptides and/or that post-transcriptional processing of these RNA may operate differently in various tissues and among different species. Further studies are now in progress to verify the biosynthesis of insulin and/or insulin-like polypeptides and to investigate whether or not these mRNA's with size larger than 630 nucleotides represent precursors of proinsulin mRNA or mRNA for other species of proteins.

Previous study in this laboratory has presented evidence that in olfactory bulb slices, insulin may modulate the function of dopamine receptors with regard to the production of cAMP (Barbaccia et al., Regulatory Peptides: From Molecular Biology to Function, pp. 511-518, 1982). It is conceivable that insulin or insulin-like peptides synthesized in the brain may function as a neuromodulator or cotransmitter for a known transmitter such as dopamine. It is also possible that insulin and/or insulin-like polypeptides are nerve trophic factors required for the maturation of neurons. Further investigation is required to clarify these possibilities. This study should lead to a better understanding of the role of

insulin and/or insulin-like polypeptides in the CNS and their relevance to keeping the functional equilibrium of our mental state.

Publication:

Lee, C.H., Lin, B.-C., Costa, E., and Chuang, D.M.: Detection of mRNA species in the brain and other tissues of which hybridize with the proinsulin gene. Fed. Proc. 43: 1796, 1984.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 01579-01 SMRP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of an Endocoid for the 5-HT₂ Recognition Site

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: B.L. Roth Guest Researcher SMRP NIMH

Others: D.M. Chuang Chemist SMRP NIMH

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COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Monoclonal Antibody Group

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TOTAL MAN-YEARS

1.8

PROFESSIONAL:

1.8

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This study concerns an investigation of the role of a putative endogenous ligand for the 5-HT₂ recognition site. We have identified a peptide isolated from bovine brain which appears to specifically inhibit the binding of 3H-ketanserin to the 5-HT₂ recognition site. This peptide has a molecular weight of about 6,000 daltons and specifically inhibits the binding of 3H-ketanserin and 3H-mianserin (another 5-HT₂-specific ligand) but it does not inhibit the 3H-imipramine or 3H-dihydroalprenolol (a β -adrenergic specific ligand) binding. Incubation of active peptide fractions with a protease (either trypsin or pronase) diminishes the activity of the peptide. The peptide has been partially purified by ion-exchange chromatography (carboxymethyl-sephadex), molecular sieve chromatography (Biogel P-10), reverse-phase chromatography (ODS) and high pressure liquid chromatography (reverse phase C8). As little as 3 g of partially purified material inhibits greater than 50% of specific 3H-ketanserin binding. We are currently testing the biologic activity of this peptide by ascertaining its effects on adenylate cyclase activity and the effect of the peptide on 5-HT stimulated phosphatidylinositol hydrolysis. Since 5-HT₂ recognition sites are down-regulated by chronic antidepressant treatment in experimental animals this putative endocoid might be involved in the therapeutic effects of antidepressants.

Project Description:

Data from radioligand binding studies suggest that there might exist at least two classes of serotonin (5-HT) recognition sites in mammalian brain. These sites have been designated 5-HT₁ and 5-HT₂ and show differential pharmacologic specificity. In brief, the 5-HT₁ site binds 3H-5-HT with high affinity (nM Kd), is regulated by guanine nucleotides and may be coupled to adenylate cyclase in the hippocampus. The 5-HT₂ site binds 5-HT with somewhat lower affinity (0.02 mM Kd), is not regulated by guanine nucleotides and binds certain 5-HT antagonists (ketanserin, mianserin) with very high affinity (nM Kd). In addition, the 5-HT₂ recognition site appears to mediate certain "behavioral" and peripheral vascular (e.g. vascular contraction) effects caused by 5-HT.

In studies performed previously in this laboratory with 3H-mianserin and 3H-ketanserin there was the suggestion that these two compounds might be labelling distinct recognition sites. Since pharmacologic manipulations which would be expected to result in supersensitivity of 5-HT₂ recognition sites (e.g. lesioning of raphe neurons) affected only the 3H-mianserin recognition site, it was suggested that 5-HT might not be the major endogenous ligand for the 3H-ketanserin site. Accordingly, we initiated a search for an endocoid for the 5-HT₂ recognition site.

Routinely, 1-3 kg of bovine forebrain are homogenized in 0.1 M acetic acid, centrifuged to remove insoluble proteins, and applied to a CM-Sephadex column. Fractions are assayed for inhibition of 3H-ketanserin binding and those fractions exhibiting the highest specific activity (ketanserin equivalents/mg protein) are combined. The active fractions are found to be eluted from the CM-Sephadex column with NaCl between 0.05 and 0.1 M NaCl. The fractions were then applied to a Sep-Pak and eluted with 70% acetonitrile; the resulting material is applied to a Biogel P-10 column. One major active fraction (MW approx. 6000 daltons) and a few minor active fractions are collected and then applied to a C-8 reverse phase HPLC column. Preliminary studies suggest the feasibility of continuing the purification by a combination of reverse phase and ion exchange HPLC.

With the most active fractions as little as 1-3 micrograms of partially purified peptide inhibits at least 50% of 3H-ketanserin specific binding. In preliminary studies specificity for the 5-HT₂ recognition sites labelled with either 3H-mianserin and 3H-ketanserin was noted. There was little specific inhibition of β -adrenergic, imipramine or 5-HT₁ binding to rat cortical membranes.

We have also assessed the protease sensitivity of the partially purified peptide. As expected, preincubation of the peptide fraction with either pronase (a non-specific protease) or trypsin partially abolished the inhibitory activity.

We have also been investigating the interaction of this peptide with the 5-HT stimulated adenylate cyclase in a clonal cell line NCB-20 (a neuroblastoma-hamster brain cell hybrid). In this system, ketanserin specifically blocks the 5-HT stimulated adenylate cyclase suggesting that the 5-HT₂ receptor is involved in this event. We are currently testing the partially purified peptide to determine whether it acts as an agonist or antagonist for this system. We have found that the peptide inhibits 3H-ketanserin binding to cell membranes suggesting that it interacts with the 5-HT₂ recognition sites on these cells as well as in the brain.

We are also investigating the 5-HT stimulated hydrolysis of phosphatidylinositol. In this system, 5-HT stimulates the production of 3H-inositol phosphates in cortical minces prelabelled with 3H-inositol provided the incubations are performed in the presence of 10 mM LiCl. Since this peptide might function as an agonist or antagonist for 5-HT₂ receptors, this system might provide a further test for the biological activity of this peptide.

These studies, therefore, suggest that there might be a peptide endocoid for the 5-HT₂ recognition sites. The ultimate purification to homogeneity and elucidation of amino acid sequence of this peptide will facilitate further studies (e.g. production of monoclonal antibodies and eventual cloning of the gene). Since 5-HT₂ recognition sites are down-regulated by antidepressant treatment in experimental animals, this putative endocoid may be involved in the affective disorders. Studies of the structure and function of the endocoid should increase our understanding on the mechanisms that are involved in the therapeutic effects of antidepressants.

Publication:

Roth, B.L., Nakaki, T., Chuang, D.M., and Costa, E.: Evidence for an endocoid for the 5-HT₂ recognition site. In Lal, H. (Ed.): Proceedings of the First International Endocoid Symposium. New York, A.R. Liss, Inc., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
 NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01580-01 SMRP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Role of Neuropeptides Derived from Proopiomelanocortin

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	W. Fratta	Visiting Scientist	SMRP	NIMH
Others:	H.-Y.T. Yang	Section Chief	SMRP	NIMH
	E. Costa	Lab Chief	SMRP	NIMH

COOPERATING UNITS (if any)

None

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.9	0.9	0

CHECK APPROPRIATE BOX(ES)

(a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The biological activity and the possible physiological role of γ -MSH peptides, which share a common precursor both in pituitary and brain with endorphins and ACTH-MSH like peptides, has been investigated. We have found that the peptide γ 1-MSH, which is the endogenous amidated form of γ -MSH, has a biological profile which resemble that of an opiate agonist. In fact, we found that γ 1-MSH inhibited the electrically induced contractions of guinea pig ileum myenteric plexus-longitudinal muscle. This effect was reversed by naloxone. Furthermore γ 1-MSH potentiated the effect of beta-endorphin and leu-enkephalin in this muscle preparation. In rat brain membrane, γ 1-MSH specifically displaced 3H-dihydromorphine, 3H-ethylketocycloazocine and 3H-D-Ala2-D-Leu5-enkephalin binding with IC-50 of around 10-7M. Moreover the opiate agonistic activity of γ 1-MSH can be effectively antagonized by ACTH(1-24), α -MSH and ACTH(4-10). These results could indicate that γ 1-MSH may have a role as synergistic modulator of endorphinergic transmission and ACTH-MSH may in contrast, have modulating effect on this transmission by antagonizing beta-endorphin or γ 1-MSH opioid-like activity.

Project Description:

In pituitary and several brain structures, the natural processing of proopiomelanocortin leads to the formation of three distinct functional families of neuro-peptides, endorphins, ACTH, α - and δ -MSH peptides. Although ACTH and MSH neuro-peptides elicit a wide spectrum of biological activity when given intraventricularly, their possible physiological role is still unclear. Though several functional roles have been proposed for ACTH-MSH peptides, a number of lines of independent investigation point out clearly that these peptides could act as endogenous opiate antagonists. In fact ACTH-MSH peptides antagonize analgesia elicited by the release of endogenous opioids or by the injection of opiates. Hence these peptides are natural candidates for an active role in opiate tolerance and in the syndrome triggered by opiate withdrawal. The mechanism of action is still unknown. Very little is known on the biological properties and possible physiological role of the δ -MSH peptide family. We have recently found that the endogenous amidated form of δ -MSH, namely δ 1-MSH, has a biological profile which resemble that of an opiate agonist. In fact, we found that δ 1-MSH inhibits the electrically induced contractions in the guinea pig ileum myenteric plexus-longitudinal muscle. This effect can be reversed by naloxone in a dose dependent manner. Furthermore δ 1-MSH potentiated the depressant effect of either β -endorphin or leu-enkephalin in this preparation. In contrast neither ACTH(1-24) nor α -MSH or ACTH(4-10) act as agonists on opiate receptors. However, ACTH(1-24) added to the bath either before or after δ 1-MSH inhibits in dose related manner the depressant effect of δ 1-MSH on electrically stimulated contraction of the guinea pig myenteric plexus. α -MSH and ACTH(4-10) displayed a similar antagonistic action but with less potency. This suggests that the amino acidic sequence 4-10 of ACTH which is contained also in α - and β -MSH seems to be essential for this action as well as for most of the non-hormonal central actions of ACTH. In rat brain membranes δ 1-MSH specifically displaces 3 H-dihydromorphine, 3 H-ethylketocycloazocine and 3 H-D-Ala²-D-Leu⁵-enkephalin binding with IC_{50} ranging from 10^{-7} to 5×10^{-7} M.

On the basis of these results the possibility emerges that δ 1-MSH may function as synergist of the opioid-like actions of endorphins while the ACTH-MSH opiate antagonistic activity might be due to either an inhibition of endorphinergic transmission or an inhibition of δ 1-MSH activity or both. Thus the proopiomelanocortin containing neurons could offer an interesting model of chemical signal interactions due to the coexistence of three neuromodulators in the same axon. It is of interest now to further investigate the interplay of these three families of peptides by extending the work on "in vivo" animal models both behaviorally and biochemically in order to infer on the molecular mechanism of action.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 01581-01 SMRP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Acetylcholine Turnover in Mouse Salivary Gland

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: C. Eva Visiting Fellow SMRP NIMH

Other: J.L. Meek Pharmacologist SMRP NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Preclinical Pharmacology

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High Pressure Liquid Chromatography Group

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TOTAL MAN-YEARS:

1.9

PROFESSIONAL:

1.9

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The cholinergic parasympathetic input to the salivary glands of cat, rat, and presumably mouse contain both acetylcholine (ACh) and vasoactive intestinal peptide (VIP). We wished to use this gland as a model for studying the functional interrelationships of such a coexistence of neurotransmitters. Although it is known that cholinergic drugs regulate release of both VIP and ACh in salivary gland, it was not known what the effects would be of VIP on the cholinergic input in vivo. In order to examine this question we developed a new technique for measurement of acetylcholine content, and used this method to measure turnover in mice infused with VIP, or injected with a muscarinic agonist (pilocarpine) or antagonist (atropine). Pilocarpine and VIP both decreased ACh turnover in mouse salivary gland; atropine increased turnover rate. The data suggest that there is a feedback loop, possibly neuronal in nature that regulates cholinergic activity. By changing postsynaptic receptor function, VIP apparently participates in the feedback regulation of ACh metabolism.

Project Description:

There are many compounds which are putative cotransmitters with acetylcholine (ACh) in various regions of brain and the peripheral nervous system. In order to study some aspects of colocalization, we chose to study a pathway in which the degree of coexistence approaches 100%: the salivary gland. VIP (vasoactive intestinal peptide) has been shown histochemically to be present in nearly all the cholinergic terminals in the rat and cat salivary gland. In cats, cholinergic drugs regulate release of both VIP and ACh, presumably via a feedback loop. We wished to perform the reverse study: to examine whether VIP modifies the ACh function.

The METHODOLOGY EMPLOYED involved development of a new simple, sensitive, selective technique for measurement of acetylcholine content based on HPLC. Turnover of ACh was estimated by infusing mice with ^3H -choline and measuring the incorporation into ^3H -ACh.

Major Findings:

1. A new method for measurement of ACh by HPLC was developed. The method involves adsorbing choline oxidase and cholinesterase on an ion exchange cartridge for use as post-column reactor. The enzymes convert Ch and ACh in the reverse phase column effluent to peroxide which can be monitored electrochemically.
2. After constant rate infusion of ^3H -choline into the mouse tail vein, the formation of ^3H -acetylcholine can be readily detected in the salivary gland. ACh turnover can be calculated using models previously developed in this lab.
3. Acetylcholine turnover is decreased after subcutaneous injection of a muscarinic agonist (pilocarpine), and increased after injection of a muscarinic antagonist (atropine).
4. VIP also decreases ACh turnover. This finding shows that VIP, a putative cotransmitter with acetylcholine is able to modify cholinergic function. The data suggest that there is a feedback mechanism operating on the presynaptic system that is triggered by receptor occupancy.

Proposed Course:

We wish to establish whether the VIP action that we observed is via a neuronal feedback loop, or instead via a local loop within the gland. ACh turnover will be measured in mice with decentralization of the parasympathetic input to the salivary gland. The effects of muscarinic antagonists and VIP will again be studied. Decentralization should decrease ACh turnover rate; atropine (a muscarinic antagonist) is predicted to now have no effect, and if VIP action is normally via a neuronal feedback loop, VIP should have no effect in the decentralized animals, either on normal ACh turnover, or that stimulated by atropine.

Significance:

Many, if not most, neurons contain more than 1 active releasable compound that may be involved in interneuronal communication. It is of great importance that we understand how these compounds interact. The mouse salivary gland allows such an analysis to begin. Many approaches are possible, including studies of VIP

antagonists, time course of VIP effects on turnover vs. VIP half-life, duration of receptor activation vs. effects on function that will enable us to better understand the fundamental mechanisms operative in neuronal communication.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 01582-01 SMRP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Effects of Estrogens on GABAergic Systems in the CNS

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: F. Nicoletti Guest Researcher SMRP NIMH

Other: J.L. Meek Pharmacologist SMRP NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

High Pressure Liquid Chromatography Group

INSTITUTE AND LOCATION

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TOTAL MAN-YEARS:

0.7

PROFESSIONAL:

0.7

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Estrogens affect brain dopaminergic systems, as shown by their ability to potentiate haloperidol-induced catalepsy in rats, to prevent apomorphine-induced stereotypies in rat, and ameliorate tardive dyskinesias induced by prolonged DOPA administration in man. The striato-nigral GABAergic system not only subserves the feedback regulation of nigro-striatal dopaminergic neurons, but also is considered the main output system for dopamine related messages of striatal origin. Repeated estradiol benzoate administrations reduce both nigral and striatal GABA concentration, and decreases the rate of GABA accumulation in the nigra induced by gabaculine, a GABA-transaminase inhibitor. This decrease of GABA turnover may be operative in the anti-dyskinetic action of estrogens in man.

A separate GABAergic system projects from the arcuate nucleus of the hypothalamus to the external layer of the median eminence. GABA released from this tuberoinfundibular pathway into the portal circulation acts on pituitary receptors to inhibit prolactin release. Estrogens are involved in prolactin regulation. We have found that repeated, but not a single administration of estradiol benzoate increase anterior pituitary GABA content, dramatically. Probably this is associated with an increase of GABA release from the tuberoinfundibular system. Consistent with this hypothesis, repeated estradiol benzoate administration increases the Vmax of glutamate decarboxylase (the enzyme which forms GABA) in the median eminence, although the Km for the substrate (glutamate) is not changed.

Project Description:

Considerable evidence supports an endocrine modulation of brain neuronal activity. Studies of hormone-transmitter interactions might provide a better understanding of such interactions. The present study is aimed at the action in male rats of estrogen administration on GABAergic activity in two systems: the striato nigral pathway and the pituitary, where GABA is not formed locally, but it is taken up from portal circulation where it is released from hypothalamus. The methodology employed involved use of HPLC to measure GABA. It is assumed that pituitary GABA content reflects the rate of GABA release into the portal circulation. We also measured kinetic parameters of glutamic acid decarboxylase (GAD), the enzyme which forms GABA in the median eminence. In the substantia nigra, we measured GABA content, GABA accumulation after inhibition of its breakdown by gabaculine, and kinetic studies of GAD.

Major Findings:

1. Administration of estradiol benzoate to rats for 2 weeks increased GABA concentration in pituitary by 6-10 fold. A single injection 4 hr before killing had no effect on GABA concentration.
2. Repeated estradiol treatment increased the V_{max} of GAD in the median eminence by 60%, but had no significant effect on the K_m .
3. Estrogen treatment decreased GABA content in the striatum and substantia nigra.
4. In animals injected with gabaculine into the substantia nigra, the accumulation of GABA was less in estrogen pretreated group than in controls.
5. GAD activity was significantly decreased in substantia nigra of estrogen-treated rats.

Proposed Course:

Since the effects of estrogens on GABA in the pituitary might be involved in a feedback control of prolactin release, blood prolactin content will be determined and correlated with changes in GABA content. Other treatments which alter prolactin will be tested both on prolactin level, and pituitary GABA: sulpiride (a dopamine antagonist), implantation of donor pituitaries, and acute administration of bovine prolactin.

Significance:

Estrogens have been implicated in the control of extrapyramidal function in experimental animals and in man. Tardive dyskinesias are more common in postmenopausal women, and estrogens are successful in the therapy of neuroleptic or L-DOPA induced dyskinesias. This study provides us with information on the basic mechanisms underlying the anti-dyskinetic activity of estrogens.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01583-01 SMRP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Receptors for Excitatory Amino Acid Neurotransmitters

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

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Other: A. Guidotti Section Chief SMRP NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Preclinical Pharmacology

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TOTAL MAN-YEARS:

0.1

PROFESSIONAL:

0.1

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects
 (a1) Minors
 (a2) Interviews
 (b) Human tissues
 (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Activation of receptors for endogenous excitatory amino acids have been shown to bring about an increase in cGMP formation in cerebellum. We have now characterized the cGMP response to various excitatory decarboxylic amino acids in 8 day old primary cultures of rat cerebellar granule cells. A specific increase in cGMP content following the addition of kainic acid to the cultures has been found. This observation will be a departing point to study interactions among kainic acid, aspartates, and glutamate recognition sites at the cellular level, and to characterize whether kainic acid receptors have a physiological role or participate in neurodegenerative disorders.

Project Description:

Neuropharmacological studies have suggested the existence of 3 different classes of postsynaptic receptors for endogenous excitatory dicarboxylic amino acids. These receptors are selectively activated by N-methyl-D-aspartate (NMDA), kainate (KA) and quisqualic acid (QA) which stimulate cyclic GMP formation in cerebellar slices (Nature 298:757, 1982). We have now characterized the cGMP response to various excitatory dicarboxylic amino acids in 8 day old primary cultures of rat cerebellar granule cells.

We found that KA produces a 5-fold increase in cGMP content, which is maximum after 1 min (ED_{50} $5 \times 10^{-5} M$). This increase is not due to an inhibition of the phosphodiesterase activity by KA since maximally inhibitory concentrations ($5 \times 10^{-4} M$) of 3-isobutyl-1-methylxanthine, an inhibitor of the phosphodiesterases, is able to potentiate the cGMP increase by KA. KA up to $10^{-4} M$ does not have a cytotoxic effect and fails to increase the cAMP content of cerebellar granule cells. The increase of cGMP is specific for KA since NMDA up to $10^{-2} M$, QA up to $10^{-3} M$, N-acetyl-aspartyl-glutamate up to $5 \times 10^{-4} M$ and glutamic acid (GLU) up to $10^{-2} M$ fail to increase cGMP content. The antagonists of these excitatory compounds, like 2-amino-5-phosphovaleric acid (PVA) and D-aminoadipate (DAA), which are selective antagonists of NMDA, glutamic acid diethyl ester, a selective antagonist of QA and GLU, up to $10^{-4} M$ do not affect the increase in cGMP content elicited by KA.

Moreover, $10^{-6} M$ bicuculline, a GABA_A receptor blocker, fails to alter the KA induced increase of cGMP content. These data suggest the existence of a selective relationship between KA receptor and cGMP. Since guanylate cyclase is a soluble enzyme the understanding of the molecular mechanisms of this interconnection between KA and guanylate cyclase is not easy. Probably an interpose second messenger brings about the activation of guanylate cyclase. An understanding of this process can provide more information on the physiological role of excitatory amino acids and the pathology related to the stimulation of receptors by excitotoxic drugs.

We are presently studying the structure activity requirement for the stimulation of the receptor occupied by kainic acid and the molecular mechanism involved in the activation of guanylate cyclase.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 01584-01 SMRP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Noncompetitive Interactions Between Mu- and Delta-Opiate Receptors In Vitro

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

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TOTAL MAN-YEARS:

0.8

PROFESSIONAL:

0.8

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Previous work suggested the existence of an opiate receptor complex, consisting of adjacent and interacting mu- and delta-binding sites. This hypothesis was based on the finding of apparent noncompetitive interactions between mu and delta binding sites. This project focused on a more detailed study of apparent noncompetitive interactions. Using rat brain membranes, and in vitro ligand binding methods, the interaction of mu ligands with 3H-D-ala2-D-leu5-enkephalin binding to delta receptors was analyzed with computer curve fitting methods. The results showed that 3H-D-ala2-D-leu5-enkephalin labeled two binding sites, and that mu ligands were competitive inhibitors at the higher affinity binding site, and noncompetitive inhibitors at the lower affinity binding site. Similarly, it was shown that the opiate antagonist, 3H-naloxone labeled two binding sites, mu and kappa receptors, and the leucine-enkephalin was a noncompetitive inhibitor at the mu binding site, but a competitive inhibitor at the kappa binding site. These results support the existence of an opiate receptor complex consisting of interacting mu and delta binding sites. Future work will explore several issues, including the existence of a kappa binding site in the receptor complex, as well as the physiological function of this receptor in light of the many chemical signals generated by several molecular forms of opiate peptides which coexist and are presumably coreleased from enkephalinergic terminals.

Project Description:

Previous work indicated the possible existence of an opiate receptor complex consisting of adjacent and interacting mu and delta binding sites. This hypothesis was based upon the finding that mu ligands were apparent noncompetitive inhibitors of delta receptor binding, and that delta ligands were apparent noncompetitive inhibitors of mu receptor binding. This project was undertaken to examine the possibility of noncompetitive interactions in greater detail and to explore the relationship of this model to other models of opiate receptors.

Using sophisticated methods of experimental design and data analysis, the interaction of mu ligands with $^3\text{H-D-ala}^2\text{-D-leu}^5$ -enkephalin binding to membranes of rat brain was studied. The results indicated that mu ligands distinguished two classes of delta binding sites: mu-competitive and mu-noncompetitive. Thus, mu ligands were shown to be competitive inhibitors at the higher affinity $^3\text{H-D-ala}^2\text{-D-leu}^5$ -enkephalin binding site, and noncompetitive inhibitors at the lower affinity $^3\text{H-D-ala}^2\text{-D-leu}^5$ -enkephalin binding site.

To explore the relationship of the two delta binding sites to previously anatomically defined type I and type II opiate receptors, the distribution of the mu-competitive and mu-noncompetitive delta binding sites were separately visualized using autoradiographic techniques and the site directed alkylating agents BIT and FIT to selectively eliminate the mu-noncompetitive and mu-competitive binding sites, respectively. The results showed that the mu-competitive and mu-noncompetitive $^3\text{H-ala}^2\text{-D-leu}^5$ -enkephalin binding sites were synonymous with type II and type I opiate receptors, respectively.

A reasonable interpretation of these results is that $^3\text{H-D-ala}^2\text{-D-leu}^5$ -enkephalin labels two binding sites in vitro: the mu-noncompetitive binding site being that delta binding site of the opiate receptor complex, and the mu-competitive binding site being a 'pure' delta binding site not associated with the receptor complex.

To ascertain whether there are two classes of mu receptors, one associated with the receptor complex, and the other not, two lines of investigation were initiated. Using $^3\text{H-naloxone}$ to label brain membranes, quantitative studies of the interaction of mu and delta ligands with $^3\text{H-naloxone}$ binding showed that this opiate antagonist labeled two binding sites, a mu and kappa site, respectively, and that delta ligands are noncompetitive inhibitors of the mu binding site. Thus no evidence for a mu binding site independent of the receptor complex came out of this study.

However, using the opiate agonist, $^3\text{H-oxymorphone}$, and the site directed irreversible and mu-selective alkylating agent beta-FNA (beta-funaltrexamine), membrane binding studies demonstrated that beta-FNA distinguished two classes of mu binding sites. Autoradiographic studies confirmed this, in that the FNA-sensitive and FNA-insensitive binding sites were distributed differently across regions of the brain. Whether or not the FNA-sensitive and FNA-insensitive mu binding sites represent mu receptors associated and not associated with the receptor complex remains to be determined.

The proposed course of this project is to:

1. Further study the effect of beta-FNA so as to define the pharmacological and physiological significance of the two mu binding sites.
2. Study the effect of the opiate alkylating agent, naloxonazine, so as to explore the relationship of this model to that proposed by Pasternak et al. (Life Sci. 27: 1185, 1980).
3. Explore the hypothesis that there might be a kappa binding site in the opiate receptor complex.
4. Test the hypothesis that the interactions of opiate peptides with the receptor complex can predict their ability to either potentiate or antagonize narcotic-induced analgesia.
5. Using selective site-directed alkylating agents, to explore on the molecular level which intramembrane signaling system is utilized by which class of opiate receptor.
6. Study the effect of opiate-dependence and withdrawal on the functions of the opiate receptor complex.

Significance of this study for biochemical research:

The role of neuropeptides in the pathogenesis of mental health remains to be defined. The opiate receptors and their endogenous peptide ligands are the best studied of the CNS neuropeptide systems, and thus serve the important role of a model system. As studies progress in the opiate field, the trail is blazed for the study of other peptidergic systems in the brain. A further understanding of the opiate receptors may lead to a greater understanding of the mechanisms involved in tolerance and dependence, and thus more effective treatments for those individuals addicted to the opiate drugs.

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