ANNUAL REPORT

Division of Intramural Research Programs
National Institute of Mental Health

October 1, 1984 - September 30, 1985

VOLUME II PART 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

NATIONAL INSTITUTE OF MENTAL HEALTH (U.S.)

OD

October 1, 1984 - September 30, 1985

VOLUME II PART II

INDIVIDUAL PROJECT REPORTS

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NATIONAL INSTITUTE OF MENTAL HEALTH

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02242-01 NPB

PERIOD COVERED

October 1, 1984 through September 30, 1985

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

Consent Rates and Informed Consent in Schizophrenia

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

Llewellyn B. Bigelow, M.D., Associate Clinical Director for Research at Saint Elizabeths Hospital, IRP, NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.20	1.00	.20

CHECK APPROPRIATE BOX(ES)

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

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

Actual consent rates for non-therapeutic investigational procedures were compared to hypothetical consent rates in two control groups. The principle finding was that there is no major difference between the control groups hypothetical consent rate and the actual consent rate experienced in our research subjects.

Project Description:

Objectives: To begin accruing empirical data surrounding the issue of intormed consent in research with schizophrenic subjects.

Methods Employed: A two dimensional questionnaire was designed asking respondents to indicate it they thought they would consent to five different procedures: (1) a 24-hour urine sample; (2) multiple venipunctures for a neuroendocrine study; (3) appear on a Public Television program; (4) electroretinogram and; (5) lumbar puncture.

The second dimension of the questionnaire posed different circumstances under which the control subject was asked to participate. There were four posited circumstances: (1) as part of a random sample without compensation; (2) as part of a random sample with compensation; (3) having a serious illness whose cause is unknown and; (4) a family member having a serious illness whose cause is unknown.

All the procedures listed were presented solely as research designed to gain understanding about the illness and were without direct therapeutic benefit.

The control groups were: (1) attendees at a conference on informed consent in mentally disabled persons and; (2) research staff in this program.

The "experimental" group data was obtained from chart review of actual consent rates in 50 consecutive participants in this in-patient research program. These subjects are moderately to severely disabled persons with chronic schizophrenia who have enrolled voluntarily.

Findings: There was no substantive difference between consent rates actually experienced on our research wards and the hypothetical consent rates expressed by the two control groups under condition 3; having a serious illness whose cause it now known. Circumstances also had a strong effect on the control's consent. The most dramatic was found for lumbar puncture where no control indicated agreement to be part of a random sample, paid or unpaid. Their 63% consent rate condition 3 was not significantly different from the patient's rate of 56%.

Significance to Mental Health Research: The bulk of meaningful research on the etiology of schizophrenia falls into the category of "greater than minimal risk without the prospect of direct benefit but designed to yield information about the patient's illness." (This category was defined by the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research). Such research is always potentially controversial, with some claiming that lacking the capacity for fully informed consent such work should not go forward.

This study is the first to provide actual empirical evidence that even patients with chronic schizophrenia can make choices regarding participation in research. Most importantly, this study demonstrates that the consent rates in the patients are similar to the hypothetical rates found for the same procedures in two different "normal" populations.

Proposed Course of Project: We will continue to collect data on consent rates for the various research protocols active in the Branch. As the sample size increases we will be able to ask more discreet questions about factors that may effect the consent process. The

relationship of subtype, other biological indices such as cerebral blood flow and results of psychological testing may all be germane to this process.

Publications:

Reiss, D., Karson, C.N., Bigelow, L.B. and Wyatt, R.J.: Specific links between psychotics' symptoms and staff's feelings. Psychiatry Res. 11:237-250, 1984.

Bigelow, L.B., Kirch, D.G., Braun, T., Korpi, E.R., Wagner, R.L., Zalcman, S. and Wyatt, R.J.: Absence of relationship of serum haloperidol concentration and clinical response in chronic schizophrenia: A fixed dose study. Psychopharmacol. Bull. 21:66-68, 1985.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: ZO1 MH 02243-01 NPB

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Visual Hallucinations and the Visual Cortex in Patients with Schizophrenia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Haim Stefan Bracha, M.D., Medical Staff Fellow, NPB, IRP, NIMH

Dr. Jon Currie, National Eye Institute, NIH; Dr. Robert Wurtz, National Eye Institute, NIH; Dr. Craig N. Karson, Staff Psychiatrist, NPB, IRP, NIMH; Dr. Owen Wolkowitz, Section on Clinical Studies, Clinical Neurosciences Branch, NIMH; Dr. Fernando Cabrera, Saint Elizabeths Hospital; Dr. Richard Jed Wyatt, Chief, Neuropsychiatry Branch, IRP, NIMH

COOPERATING UNITS *(if any)*
Saint Elizabeths Hospital; National Eye Institute, NIH;
Section on Clinical Studies, Clinical Neurosciences Branch, NIMH

LAB/BRANCH
Neuropsychiatry Branch

SECTION
Section on Clinical Brain Studies

INSTITUTE AND LOCATION
NIMH, Saint Elizabeths Hospital

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.20	.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.)*

We have examined lifetime prevalence of visual hallucinations in research subjects with schizophrenia in a retrospective study. We have found a lifetime prevalence of 32% and of those interviewed, 56%. Other correlates of visual hallucinations are currently being studied.

Project Description:

Objectives: Little has been written about visual hallucinations in chronic schizophrenia. Although two recent reports from around the world give the prevalence of visal hallucinations in schizophrenia to be 43% in Kenya and 62% in Saudi Arabia, these reports are regarded as cross-cultural anomalies. We therefore first examined the prevalence and phenomenology of visual hallucinations in a large number of research subjects with the diagnosis of chronic schizophrenia.

Methods Employed: Patients met DSM III and RDC criteria for chronic schizophrenia. We used the term "visual hallucinations" as defined in the DSM III glossary of technical terms reviewed charts of 100 discharged patients and reviewed charts and interviewed consecutively admitted subjects.

Major Past Findings: We have recently reported that visual hallucinations in 38 right handed patients with schizophrenia were significantly more frequent in the visual hemifield processed by the dominant visual cortex. This is in agreement with several investigators who have reported that schizophrenic patients have significantly longer response latencies to visual stimuli presented to the right visual hemifield and suggests either a greater involvement of the dominant hemisphere in some patients with schizophrenia or a subtle asymmetry of visual attention in these patients.

New Findings: 1) In our retrospective chart review of 100 patients discharged from the NIMH with a diagnosis of schizophrenia, we have found clear documentation of past or present visual hallucinations of 32% of the patients. 2) Of the 43 prospectively interviewed consecutively admitted subjects with a research diagnosis of chronic schizophrenia, 24 patients, or 56% had unequivocal past or present visual hallucinations.

Our findings suggest that visual hallucinations are more common in chronic schizophrenia then generally believed. Our findings also suggest that contrary to current thinking the prevalence of visual hallucinations in schizophrenia is similar a cross cultures.

Significance to Mental Health Research: Neuro-opthalmological abnormalities are of interest for two reasons, 1) in general they are easily quantified and studied, and 2) since the neurobiology of the visual system is relatively well understood, these abnormalities may provide information concerning the neurobiology of schizophrenia. Since visual hallucinations are rare in affective illness (lifetime prevalence of 9% and 1% in bipolars and unipolars, respectively) their presence in a "functional" psychosis may help in differential diagnosis.

Proposed Course of Project: Three related studies are expected to advance this initial work. They are 1) tachistoscopic study of visually hallucinating versus non visually hallucinating patients with schizophrenia; and 2) an investigation of spontaneous lateral eye movements in visually hallucinating versus non visually hallucinating patients with schizophrenia, a controlled study.

Publications:

Bracha, H.S., Cabrera F., Karson, C. and Bigelow, L.B.: Laterality of visual hallucinations in schizophrenia: Increased frequency in the right visual hemifield. Biol. Psychiatry, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02244-01 NPB

PERIOD COVERED

October 1, 1984 through September 30, 1985

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

Behavioral Effect of Neurotoxic Substances and Their Neurochemical Correlates

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

Jean Lud Cadet, M.D., Staff Psychiatrist, NPB, IRP, NIMH

Dr. William J. Freed, Chief, Section on Preclinical Neurosciences, NPB, IRP, NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Preclinical Neurosciences Section

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.20	.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.)*

We are studying the toxic effects of various substances on the behavior of rodents. These will be carried out in conjunction with biochemical and histological evaluation of neurochemical systems that may be involved in the behavioral changes. In some cases, attempts will be made to protect against these effects by the use of antioxidants.

PHS 6040 (Rev. 1/84) GPO 904-917

Project Description:

Objectives: The evidence accumulated so far suggests that neurotransmitter systems play a role in neuropsychiatric movement disorders. Parkinson's disease, for example, is thought to involve the neurotransmitter dopamine and can be clinically managed by administration of dopamine agonists and precursors. This has not, however, led to the elucidation of the cause of the disorder. Others (for example: Tardive dyskinesia) still resist pharmacological manipulation. Data from animal models of these disorders might provide essential information regarding which behavioral abnormalities might be more closely related to specific neurotransmitter dysfunction. Another purpose of these studies is to evaluate possible preventive mechanisms that may also be useful clinically.

Methods Employed: Groups of animals were injected intraperitoneally or intracerebrally with neurotoxic substances. Locomotion and stereotypic performances were evaluted after injection of various compounds (dopaminergic, serotonergic, etc.). Brain specimens were also collected for histological and biochemical correlation with the behavioral changes.

Findings: One of the neurotoxic agents, iminodipropronitrile, causes irreversible hyperactive and dyskinetic neck movements in both mice and rats. These abnormal movements are exacerbated by DA agonists and blocked by antagonists. In other experiments intrastriatal IDPN causes ipsilateral rotation to DA agonists and IP injection of IDPN causes an increase in the level of HVA. These data suggest that IDPN may be a DA receptor antagonist.

Significance to Mental Health Research: These studies provide models of movement disorders that are more easily pharmacologically manipulated. The results obtained may help in planning better treatment for patients who suffer from these movement abnormalities.

Proposed Course of Project: This project will continue and will address the question of neurotransmitter interactions and neuroanatomical structures that may be involved in this behavioral syndrome. The results of these experiments may help focus the treatment of movement disorders such as tardive dyskinesia.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02245-01 NPB

PERIOD COVERED

October 1, 1984 through September 30, 1985

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

Functional Consequences of Experimental Nerve Lesions

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

Luis de Medinacelli, M.D., Visiting Scientist, Neuropsychiatry Branch, IRP, NIMH

Susan L. Darlington, Biologist, NPB, IRP, NIMH
Dr. Richard Jed Wyatt, Chief, NPB, IRP, NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Section on Aging

INSTITUTE AND LOCATION

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.30	.80	.50

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.)*

Crush lesions were made on sciatic nerves of rats. We examined the influence on recovery of the time elapsed between successive injuries and of location and number of damaged sites. The results were assessed over a post-operative period of 2.5 months by studying tracks obtained from walking rats.

GPO 904-917

Project Description:

Objectives: We have shown in previous work that when a rat sustains two successive peripheral injuries, the second restoration of function is not as good as the first. The disruptive effect of a secondary lesion is not linked to the existence of a previous "scar." Instead, a systemic response - possibly the autoimmune reaction observed at the site of the lesion - seems responsible for the disruption of recovery. This new work has been designed in order to a) better define the time dependency of this systemic response; b) examine the impact on recovery of the distance along which the sprouts have to travel and c) determine whether in fact the local scar left by a preliminary nerve lesion could also have some influence on regrowth. We will evaluate the results exclusively in terms of functional recovery.

Methods Employed: Nerve injury. Male rats weighing 200-300 g at the beginning of the experiment were anesthetized with phenobarbital and chloral hydrate i.p. The left sciatic nerve was aseptically exposed in the thigh. Damage was made by crushing the nerve with maximum force for 1 minute in the bare jaws of a serrated hemostat. There were be approximately 10 animals per group. In "control-lesion" groups, the crush was made at one of two sites. In group I (control low), the three sciatic branches were crushed just above the popliteal vessels. In group II (control high), the nerve trunk was crushed at the level of the tendon of the m. obturator externus.

In "simultaneous-lesions" groups, some rats received one high crush and one low (group III; simult. double). In other animals (group IV; simult. quadruple), two more crushes were added between high and low sites. In group V (simult. extensive), the nerve was extensively damaged. This was achieved by crushing it for 1 minute at the high and the low sites and then making forcible contiguous crushes of 2 seconds duration between these extreme points.

In "successive-lesions" groups, all nerves were first crushed at the high site. A second lesion was made at the low site 4 days later (group VI; succes., 4d), 11 days later (group VII; succes., 11d) or 18 days later (group VIII; succes., 18d).

After surgery, all animals received one injection of penicillin in the unoperated thigh and were caged separately on soft beddings to diminish sensory-deprivation sores and resulting autophagia.

Testing. The functional condition was assessed through the use of the sciatic functional index (SFI), based on measurements made from rats walking tracks (1). Animals that engaged in post-operative autophagia were discarded. Collection and analysis of data were made by a "blind" observer with a data management system. Animals were tested pre-operatively and on post-operative day 1,4,7,11,15,18,21,25,32,39,46,53,60,67 and 74. In "successive-lesions" groups, the rats were tested as above after the first operation up to the day chosen for the second lesion. They were then tested immediately prior to surgery and the numeration of days started again at zero.

Major Past Findings: It is generally agreed that the main role of time in nerve injuries is to allow the progressive development of local or systemic changes capable of influencing sprout regrowth. Whether these changes are beneficial or not is still a matter of discussion. We have previously

demonstrated the existence of a post-traumatic change that is somewhat detrimental. We are examining its development in time. We have also hypothesized that the outcome of a nerve injury is not influenced by the distance the sprouts have to traverse before reaching their destination, but is determined essentially by events occurring in the area of damage (vulnerable regrowth zone). Distance, however, may have some effect on the pattern of recovery by scattering the sprouts along the nerve. Sprout velocity is not homogeneous. When the distance increases, the intervals among the front running sprouts and the slower growing ones become larger. Thus, recovery can be temporarily distorted, since the arrival of sprouts at their destination is highly asynchronous when the distance is great. This effect, however, does not last and disappears when the slower neurites catch up. Finally, peripheral nerves are formed of three completely distinct elements, i) cell processes, ii) fundamental tissue and iii) amorphous structures; the last two constitute the glia. In cases of injury, all three elements are affected and each in a different way. i) We have previously shown how to minimize cellular injury, i.e. damage that affects only part of the neural cell. ii) The fundamental tissue is formed mainly of Schwann cells and also of connective cells found in blood vessels and nerve sheaths. These elements sustain a tissular injury, i.e. an injury that wholly destroys cells at the site of trauma but does not directly affect the surrounding ones. Multiplication of intact cells from the borders of the lesion will ensure healing. Tissular damage is present in all nerve injuries, even those where the axons are spared. iii) The amorphous structure is mainly composed of basal lamina tubes surrounding the fibers and also of non-cellular substances of the neuropil and basal membranes forming the fascicle sheaths. These elements sustain a structural injury, i.e. an inconspicuous injury that dislocates the micro-skeleton of the nerve. Once this unique framework has been disjointed, complex techniques will be required to grossly reconstruct the original pattern. Structural injury is observed in nerve transections or avulsions but seems absent or minimal in crush lesions.

New Findings: Data are being analyzed.

Significance to Mental Health Research: We expect this research to provide information on trauma and related diseases of nervous tissue and on its regeneration.

Proposed Course of Project: The project could be completed during the next reporting period.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 02246-01 NPB

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Post-Traumatic Autoimmune Reaction In Peripheral Nerve

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

Luis de Medinaceli, M.D., Visiting Scientist, NPB, IRP, NIMH

Dr. Allen C. Church, Staff Fellow, NPB, IRP, NIMH; Dr. Yen-Nung Wang, Visiting Associate, NPB, IRP, NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH
Neuropsychiatry Branch

SECTION
Section on Aging

INSTITUTE AND LOCATION
NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.25	.75	.50

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.)*

This study was conducted to show whether local autoimmune reactions can be observed after injury to the sciatic nerve in the rat. Furthermore, we attempted to correlate the intensity of the immunological reaction with the severity of nerve damage, with the type of surgical treatment and with the degree of functional recovery.

PHS 6040 (Rev. 1/84) GPO 904-917

Project Description:

Objectives: Autoimmune phenomena have been described in some acute neuropathies, in subacute demyelinating disease, and in inflammatory polyneuritis. At present it is unclear whether these autoimmune responses play a significant role in either the etiology or the course of such disorders. The production of antibodies against the nerve components suggests that the barrier normally separating the blood from axons and myelin - i.e. the blood-nerve-barrier (BNB) -has broken down and has allowed neural antigens to leak into the circulation. Since the BNB is always disrupted or destroyed at the site of a traumatic nerve injury, leakage of nerve-associated molecules into the circulation must occur. This leakage of neural proteins, some of which are antigenic, might be expected to elicit an autoimmune response. In fact it has recently been reported that antibodies directed against gangliosides and myelin are present in the serum of mice subjected to sciatic nerve transection. To our knowledge, local modification of peripheral nerves due to post-traumatic autoimmunization have not been described and the possible implications of such processes have not been studied. We initiated the present work to determine whether, after peripheral nerve injury: (a) a local autoimmune reaction (AR) could be demonstrated in the nerve; (b) a correlation could be found between the severity of the initial damage and the intensity of the AR; (c) a correlation could be found between the type of nerve repair and the intensity of the AR; and (d) a correlation could be found between the intensity of the AR and the degree of long-term functional impairment.

Methods Employed: The study was conducted in three separate phases.

Phase I: Effect of a Single Injury: Rats were assigned to one of six treatment groups and studied using both behavioral and histological techniques. Group I (control) animals were allowed to recover without a surgical procedure. In all other groups, the left thigh was opened and the sciatic nerve trunk exposed. In group II (crush) the nerve was crushed for 1 minute at mid-thigh by maximally closing the bare jaws of a serrated hemostat. In all other animals the nerve was transected at mid thigh. One of three procedures was then used.

Surgical procedures: In some animals (group III, no repair) the reunion of the stumps was prevented. The proximal stump was pulled upward and sutured to the m. gluteus. In other animals (group IV, reconnection) the nerve was repaired with the reconnection technique. The term reconnection designates a complex method of repair that brings consistent functional recovery after nerve transection in the rat. Briefly, the nerve is first soaked in a fluid containing chlorpromazine and polyvinyl alcohol, and is sutured to a small rubber support. The preparation is cooled to 10°C and soaked in a second fluid, a modified Collin's fluid, that mimicks the intra-axonal ionic concentration. The nerve is then briefly frozen to -1.5°C, transected with a vibrating knife and immediately thawed. After approximation of the stumps, the preparation is brought to body temperature and rinsed with BSS-Plus fluid. The rubber support is removed on the 20th post-operative day.

In the remaining animals, the stumps was sutured under Ringer's fluid irrigation with 3 to 5 perineural stitches of 9/0 nylon monofilament (Prolene). On the basis of the long-term behavioral results these animals were assigned to either group V (successful suture) or group VI (unsuccessful suture).

Phase II: Effect of Two Successive Injuries at the Same Site: There were four groups of animals in this phase.

Animals in group I (control) were allowed to recover without a surgical procedure. In all other animals, the site of injury was the mid-thigh portion of the left sciatic nerve. In group II (double crush) the nerve was first lesioned by maximally closing the bare jaws of a serrated hemostat for 1 minute. The second crush was identically made 32 days later.

In group III (double reconnection) the first injury was a transection of the nerve followed by a reconnection. The second injury, 32 days later, was a complete resection of the area of the first damage. A length approximately equal to two diameters of the nerve was removed. Repair was obtained by another reconnection. To avoid additional surgical aggression in this group, the antiretraction device was not removed around the 20th postoperative day as usually done. Instead, the first antiretraction device was removed during the second procedure and the second device was kept in place until the time of sacrifice.

In group IV (double suture) the nerve was first transected and sutured under lactated Ringers' fluid irrigation with 2 to 4 perineurial stitches of 9/0 nylon monofilament (Prolene). The second injury, 32 days later, consisted of a complete resection of the area of the first damage. A length approximately equal to two diameters of the nerve was removed. Repair was then accomplished by suturing the stumps as described above.

Phase III: Effect of Two Successive Injuries at Different Locations: There were six animal groups in this phase.

Animals in group I (control) were allowed to recover without a surgical procedure. Rats in group II (control crush) received a single crush injury. Their left sciatic nerve was forcibly crushed at mid-thigh for 1 minute in the bare jaws of serrated forceps. Animals in group III (control suture) also received a single injury. Their left sciatic nerve was transected at mid-thigh with scissors and was immediately sutured under lactated Ringers' fluid irrigation with 2 to 4 epineurial stitches of 9/0 nylon monofilament (Prolene). All experimental animals received two successive peripheral nerve injuries, the second injury being made one month after the first and at a different location. The first lesion (conditioning damage) was an injury to the right median nerve. The nerve was transected above the wrist and the tip of the proximal stump was crushed over a 2 mm length. The second injury will be made 32 days later. Its site was the left sciatic nerve trunk at mid-thigh. In some rats (group IV, condit. damage + crush) the sciatic nerve was crushed. In other animals (group V, condit. damage + suture), the sciatic nerve was transected and repaired by micro-sutures .

Testing: Nerve function was evaluated by the sciatic functional index (SFI) which is based on measurements made on rats' walking tracks and is expressed in units of functional deficit. All animals were tested pre-operatively and at regular post-operative intervals. The tracks were analyzed through a sciatic index data management system with digitized input. Testing of the animals and analysis of the tracks were made by a "blind" observer.

Immunohistochemical techniques: Two and a half months after the last surgical procedure the animals were anesthetized again and the sciatic nerve trunk removed. The nerves were slightly stretched on a small piece of cardboard, placed in phosphate buffered (pH 7.4) 4% paraformaldehyde for 24 hours and then in 30% sucrose for 24 hours. The tissue was then frozen, cut longitudinally at 16 micrometers in a cryostat and mounted on subbed slides, 2-3 sections per slide. Randomly chosen sections of each specimen were stained for the presence of immunoglobulins using goat anti-rat gamma globulins (1:50) conjugated to fluorescein isothiocyanate. One slide for each animal was examined using a Zeiss photomicroscope with incident light fluorescence illumination. All slides were first

examined in a non-blind way in order to discern an eventual pattern. They were then examined again blindly by two observers and tentatively assigned to different groups. To study the specificity of staining animals were chosen at random in each of the treatment groups. From each of the resulting nerves, two slides at consecutive levels were taken. One slide was stained using primary antiserum that was preincubated in 10% whole rat serum for 48 hours in order to inactivate all anti-rat gamma globulin antibodies through binding to the rat serum gamma globulins. The other slide was processed at the same time but whole rat serum was omitted in the preincubation.

In order to obtain photographs which accurately indicate relative luminosity, all negatives and subsequent prints were produced using identical exposures and equal printing times. In this way, we obtained final pictures that will be reliably compared with one another in terms of the intensity of gamma globulin immunoreactivity.

Major Past Findings: Results from pilot experiments support the notion that nerve trauma produces leaks of neural antigens. The autoimmune response that followed this leakage appeared to be characterized essentially by humoral components. A clear association between the severity of the initial damage and the intensity of the AR seems to exist and could result from trauma-associated differences in a) the duration of antigen release; b) the quantity of antigen release and c) the quantity of autoantibodies that reach the endoneurial compartment.

New Findings: Data are being collected.

Significance to Mental Health Research: We think that this work has a clinical importance in the field of nerve tissue injuries and regeneration.

Proposed Course of Project: The duration of this project is estimated to be about one year.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 02247-01 NPB

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Prediction of Outcome of Peripheral Nerve Injuries - a Probability Model

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Luis de Medinacelli, M.D., Visiting Scientist

Robert R. Rawlings, Mathematical Statistician, Div. Biometry and Epidemiology, NIAAA, Dr. Allen C. Church, Senior Staff Fellow, NPB, DIRP, Dr. Y-N. Wang, Visiting Associate, NPB, DIRP, Dr. Richard J. Wyatt, Chief, NPB, DIRP

COOPERATING UNITS *(if any)*
Division of Biometry and Epidemiology, NIAAA

LAB/BRANCH
Neuropsychiatry Branch

SECTION
Section on Aging

INSTITUTE AND LOCATION
NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.20	.20	

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.)*

The long term functional consequences of peripheral nerve injuries are notoriously unpredictable. We hypothesized that considering the individual regrowth of the elementary components of a nerve (the neurites) rather than the global regeneration of the organ could lead to a better understanding of the mechanisms of nerve repair.

We postulated that the regrowth of any individual neurite can be defined in terms of its influence on recovery, the three main possibilities being valid, neutral and invalid regrowth. We have designed a probability model describing the prospects of regrowth for nerve composed of several types of fibers. This model is being tested in pre-determined situations to judge its validity.

PHS 6040 (Rev. 1/84) GPO 904-917

Objectives: After an injury to a peripheral nerve, the distal portion of the neurite dies and is digested by the support tissue. The proximal portion usually survives and soon produces a new extension that tends to re-establish communication between the centers and the periphery.

If the injury leaves the amorphous structures intact, the new extensions will grow in the basal lamina tubes as if in a tunnel. Because of this guiding effect, all sprouts will have a good chance of reaching their appropriate target. This is the case after crush lesions.

Conversely, transections and avulsions destroy all pathways at the site of injury. In these cases, the area of damage is the source of considerable criss-crossing and misrouting of the sprouts. There is little doubt that errors in path-finding lead to a decrease in the number of appropriate connections between the centers and the periphery and, consequently, to a decrease in the quality of function. As expected, the average functional outcome of nerve transections is unsatisfactory. There is, however, a great variability in the individual results. Identical operative procedures applied to similar injuries result in very different recoveries. We think that, in fact, this variability follows a pattern. In various studies where the sciatic nerve of the rat was transected and repaired, the ratios of satisfactory, mediocre and poor functional results were similar in all experiments, even though the number of animals in each group was small. Such a trend is also found in man after peripheral nerve surgery. Studies of either large or small groups of patients reveal a distribution of the results that does not seem strictly random. Also, distribution of results vary from one type of nerve to another. Some nerves are known to recover quite well, while injuries to others have consistently poor prognoses.

We hypothesized that a simple statistical explanation can be offered for these observations and we designed a probability model as a help in understanding some fundamental mechanisms of nerve repair.

Methods employed: We made arbitrary assumptions on basic conditions in a regenerating nerve. We hypothesize that: a) the path followed by each sprout can be defined in terms of its consequence on function; b) the number of theoretical possibilities thus determined is limited and c) clinical variations can be explained by blending all possible reinnervation patterns. We considered that there are two theoretical types of injuries, a given proportion of lost neurites, and three theoretical reinnervation prospects for regenerating neurites.

We hypothesized that from a functional point of view, regrowth of each neurite can be valid, neutral or invalid.

We also took into consideration the notion of vulnerable regrowth zone, and the theoretical number of sprouts per neurite.

As far as peripheral connections are concerned, we postulated that a) a functional connection would be achieved by any neurite growing into a pathway previously occupied by a neurite of the same denomination; b) the end organ itself is functional.

According to the type of fibers, we i) divided motor neurites into two sub-groups (we considered that a neurite of one sub-group growing into a pathway previously occupied by a neurite of the other sub-group would result in an invalid connection); ii) divided sensory fibers into four sub-groups only, corresponding to tact, temperature, proprioception and pain sensations. Based on clinical studies, we considered that a sensory fiber of one type growing into a pathway of another sensory type would result in an invalid connection; iii) consider that in the case of the autonomic system, no precision of targeting is necessary, i.e. that an autonomic neurite growing into any autonomic pathway would establish a valid peripheral connection.

Based on these assumptions, four theoretical types of nerves can be recognized. Type 1 defines either motor or sensory branches containing no autonomic fibers and going to peripheral organs that all perform the same or a synergetic function. Type 2 defines nerves composed of either motor or sensory fibers, containing a significant proportion of autonomic fibers and going to peripheral ensembles that perform the same or a synergetic function. Type 3 defines nerves composed of either motor or sensory fibers, containing autonomic fibers and branching towards peripheral ensembles that perform agonistic and antagonistic functions. Type 4 defines large, mixed nerves composed of motor, sensory and autonomic fibers and innervating peripheral ensembles that perform agonistic and antagonistic functions.

The model is described in the case of a type 4 nerve, i.e. the most complex case.

As stated above, we recognized one type of autonomic neurites (A), two types of motor neurites (M_1 and M_2) and four types of sensory neurites (S_1, S_2, S_3 and S_4). Lost neurites of any type are designated by L. The proportions of the two types of motor neurites are denoted by t_1 and t_2, such that $t_1 + t_2 = 1$. The proportions of the four types of sensory neurites are denoted by s_1, s_2, s_3 and s_4, such that $s_1 + s_2 + s_3 + s_4 = 1$. The overall proportions of autonomic, sensory and motor neurites are denoted by p_1, p_2 and p_3 respectively, such that $p_1 + p_2 + p_3 = 1$.

After injury, there is a probability of regrowth into autonomic, motor and sensory pathways as well as a probability of lost neurites (q). The total probability of regenerating neurites being valid (P_V), invalid (P_I) or neutral (P_N) is determined. The probabilities thus obtained are expected to be as follows:

$$P_V = [p_1^2 + p_3^2(t_1^2 + t_2^2) + p_2^2(s_1^2 + s_2^2 + s_3^2 + s_4^2)] \ (1 - q)$$

$$P_I = [2t_1t_2p_3^2 + 2p_2^2(s_1s_2 + s_1s_3 + s_1s_4 + s_2s_4 + s_2s_3 + s_3s_4)] \ (1 - q)$$

$$P_N = 1 - P_V - P_I$$

Other types of nerves. By placing appropriate constraints on the parameters, models for nerves type 1, 2 and 3 are being obtained.

Utilization of the model. By varying the parameters p_1, p_2, q, t_1, s_1, s_2, and s_3 over reasonable ranges of values, the effects of different factors on the resultant triplets (P_V, P_I, P_N) are being examined. To our knowledge, an atlas giving the exact proportions of the three types of fibers in each nerve does

not exist and information on this subject is still fragmentary. We think it possible, however, to use the probability model by attributing approximate values to these proportions.

Major Past Findings: To my knowledge, no model of this kind has been devised in the field of nerve injury.

New Findings: Data are being analyzed.

Significance to Mental Health Research: Unpredictable functional results are a characteristic feature of peripheral nerve surgery. This has led to various hypotheses in order to explain the erratic clinical observations. Among these hypotheses, one of the most enduring is the belief that regrowth is guided by some kind of a chemotropism. No neurotropic action, however, has ever been demonstrated to influence the regrowth of new sprouts in the peripheral system. Our model tends to demonstrate that chemotropism is not necessary and that a more parcimonious explanation suffices to explain the results. We therefore expect that this model will shed a new light on the basic mechanisms of nerve regeneration after injury and disease.

Proposed Course of Project: Development of this model will be completed during the upcoming reporting period. Use of this model will be applied in subsequent studies investigating regeneration of severed peripheral nerves.

Publications:

de Medinacelli, L., A.C. Church and Y.N. Wang. 1985. Posttraumatic autoimmune reaction in peripheral nerve: Effects of two successive injuries at different sites. Exp. Neurol., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 MH 02248-01 NPB

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Endogenous Substance Extract from Human Brain Inhibiting Neuroleptic Binding

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Anne-Marie Duchemin, M.D., Visiting Fellow, NPB, IRP, NIMH

Dr. Bruce H. Phelps, Staff Fellow, NPB, IRP, NIMH; Dr. C. David Wise, Research Chemist, NPB, IRP, NIMH; Dr. Richard Jed Wyatt, Chief, Neuropsychiatry Branch, IRP, NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH
Neuropsychiatry Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.75	.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.)*

Extract from human brain inhibited in vitro ^{3}H-spiroperidol binding. This inhibition showed a regional distribution and was specific for neuroleptic binding. The identification of this substance showed that it is represented by endogenous dopamine and a conjugate forum of this neuromediator.

PHS 6040 (Rev. 1/84) GPO 904-917

Project Description:

Objectives: Dopamine (DA) is known to have a weak affinity for the neuroleptic binding sites (in the micromolar range) while the affinity of the neuroleptics themselves is in the nanomolar range. In some other neurotransmitter systems, it has been already demonstrated that the antagonists have a higher affinity for the receptors than the natural agonist. In the case of the neuroleptics, however, the other explanation for this discrepancy could be the existence of another endogenous ligand that would occupy the antipsychotic binding sites. In fact, it has been reported that there is an inhibition of ^{3}H-spiroperidol binding by an endogenous material extracted from bovine brain and the existence of an endogenous neuroleptic in the brain has been suggested. We reproduced these experiments using human brain tissue.

Methods Employed: Human brain tissues from patients without psychiatric or neurologic illness were collected at autospy and an extraction procedure was used to prepare the brain extracts.

Then biochemistry technics were used either to quantify and characterize the inhibiting factor either to purify or identify it.

The first ones consisted of radioreceptor assays: Neuroleptic and cholinergic receptors were assayed in vitro by using binding of labeled ligand on rat caudate membranes.

The second ones consisted of chromatography procedures such as spehodex gel filtration, thin layer chromatography, and reverse phase ion-pairing liquid chromatography.

Findings: Inhibitory activity of the brain extracts was evaluated in the ^{3}H-spiroperidol binding assay. The binding inhibition showed a regional distribution with the following order: putamen > caudate > nucleus accumbens > cerebellum = frontal cortex. This observation ruled out a non-specific inhibition by salts or ethanol residue from extraction procedure. This inhibition seemed to be specific of the neuroleptic binding since the putamen extract produced a 40% and 50% inhibition of the ^{3}H-spiroperidol and ^{3}H-haloperidol bindings respectively without modifying the interaction of a non-neuroleptic ligand (^{3}H-QNB: + 3%).

Although a large portion of the endogenous DA must have been destroyed during the extraction procedure, we checked if a remaining amount of the transmitter could explain the binding inhibition, as the regional distribution of the neuroleptic binding inhibition coincided with athe DA content of these brain areas.

Thus, the brain extracts were placed on a spehadex G 10 column and eluted fractions were evaluated in the ^{3}H-spiroperidol binding assay. With putamen and caudate extracts, two peaks of binding inhibition were observed. No inhibition peaks were found with cerebellum and frontal cortex.

A series of experiments were conducted in the presence of ^{3}H-DA added at the first homogenization step of the brain extraction procedure. Aliquots of each elution fraction were tested for their tritiated content. The radioactivity eluted from the sephadex column was recovered in two parts, well correlated with the two peaks of ^{3}H-spiroperidol binding inhibition.

To identify the component present in each peak, aliquots of the fractions were chromatographed alongside DA on silica Gel thin layer chromatography plates. The migration curves of the second inhibition peak fractions were found strictly parallele to the

DA used as standard. To confirm the presence of DA in the brain extracts, the sephadex fractions were analyzed by HPLC for their DA content. DA was found present in micromolar concentration in the ^{3}H-spiroperidol binding inhibition peak fractions from caudate and putamen only. A good correlation was established between the values of binding inhibition obtained experimentally and the inhibition expected from the DA concentrations measured by HPLC.

The first ^{3}H-spiroperidol binding inhibition peak did not seem to represent authentic DA. IN fact, no detectable DA was observed in these fractions in HPLC analysis and aliquots of these fractions did not parallele DA when deposed on the thin layer chromatography plates. However, there was evidence that a DA derivative was present in these fractions since, when extract brain containing ^{3}H-DA was placed on the sephadex column, radioactivity was also eluted in the first inhibitory peak fractions.

We concluded that, using our tissue preparation technics, the inhibition of neuroleptic binding sites observed with human brain extracts, was due to endogenous dopamine.

Significance to Mental Health Research: Because dopamine has been implicated in the pathogenesis of schizophrenia, any attempt to elucidate it's mechanisms of action are highly relevant to the study of this disorder.

Proposed Course of Project: We will continue to collect and analyze data throughout the next reporting year.

Publications:

Duchemin, A.M., Phelps, B.H., Wise, C.D. and Wyatt, R.J.: Inhibition of neuroleptic binding by human brain extracts - An effect of endogenous dopamine? Eur. J. Pharmacol. 107:253-258, 1985.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 02249-01 NPB

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Ontogeny of Preprocholecystokinin mRNA and Cholecystokinin in the Rat Brain

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Anne-Marie Duchemin, M.D., Visting Fellow, NPB, IRP, NIMH

Dr. Richard Jed Wyatt, Chief, Neuropsychiatry Branch, IRP, NIMH; Dr. Tan Thau Quach, Laboratory of Preclinical Pharmacology, IRP, NIMH; Dr. Michael Iadarola, Laboratory of Preclinical Pharmacology, IRP, NIMH; Dr. Joan Schwartz, Laboratory of Preclinical Pharmacology, IRP, NIMH; Dr. R.J. Deschenes, Biochemical Dept., Purdue University, West Lafayette

COOPERATING UNITS *(if any)*

Biochemical Dept., Purdue University, West Lafayette

LAB/BRANCH
Neuropsychiatry Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.80	.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 expression of the gene coding for cholecystokinin (CCK) was studied pre- and post-natally in rat brain with a preproCCK specific cDNA probe to quantify mRNA. In addition, a c-terminal specific radioimmunoassay was used to measure CCK immunoreactive peptides (CCK-IR).

A single band of mRNA corresponding to ≃800 bases, was observed after agarose gel electrophoresis of poly(A)+ RNA isolated from brain and Northern blot hybridization with the labeled preproCCK cDNA probe. Brain preproCCK mRNA was detectable by embryonic day 14 and progressively increased to reach maximum levels two weeks after birth. It tended to decrease slightly in adult rats.

CCK-IR was undetectable at embryonic day 14. At embryonic day 20, detectable levels were measured but marked development was seen only after birth with a rapid increase between postnatal days 7 and 21. By three weeks, the concentration reached approximately 90% of the adult levels.

Specific mRNA synthesis precedes pronounced appearance of the synthetic and storage machinery needed for neurotransmitter peptide function. The pattern of the ratio of CCK mRNA:CCK-IR from embryonic day 21 to adulthood suggests that synthesis or storage of the peptide alter during development.

PHS 6040 (Rev. 1/84) GPO 904-917

Project Description:

Objectives: Several authors have studied the ontogeny of cholecystokinin (CCK) in the brain by both radioimmunoassay and by immunohistochemical analysis. Immunohistochemical analysis has shown that the first CCK cell bodies are detected at gestational day 15. The cells reach a maximum at post-natal day 10, then tend to decrease slightly in number. Marked development of CCK fibers have been seen only between post-natal days 5 and 10. A study of the ontogeny of CCK receptors in rat brain showed a very low level at birth, a maximum level at day 12 and a decline to adult level by day 26.

In this report, we have examined the expression of the CCK gene, by measuring in parallel preproCCK mRNA and CCK-IR in the rat brain during embryonic and post-natal development.

Methods Employed: Quantification of preproCCK-mRNA: PreproCCK mRNA has been detected in rat brain by Northern blot analysis using a cDNA hybridization probe complementary to CCK mRNA isolated from a rat medullary thyroid carcinoma. This probe was the Ava Xma III restriction fragment from plasmid pCK 2AB5, nick-translated with (α-^{32}p) dCTP.

RNA from rat whole brains at different ages was prepared by excreted with guanidium thiocyouate and centrifugation in cesium Ahloride-Poly(A)+-RNA was then purified by chromotography on oligo-dT-cellulose.

RNA was electrophoresed on formeldelhyde-agarose and gel paper-RNA blots were hybridized with nick-translated cDNA probe. Quantitative analysis of the resulting autoradiographs was performed by densitometric scanning.

CCK Radioimmunoassay: CCK was measured in the brain samples, using an antiserum directed against the c-terminal amide portion of CCK-8 and cross-reacting equally with sulfated and non-sulfated CCK-8. ^{125}I-CCK-8 was used as the tracer and was iodinated with the chloramine T method.

Findings: Identification of rat brain preproCCK mRNA: Northern blot hybridization of rat brain poly(A) RNA with the preproCCK cDNA probe showed that only one species of mRNA hybridized with the probe. Based on ribosomal RNA size markers, the size of the preproCCK mRNA was estimated to be 800 $\pm$ 100 bases in agreement with previous results. The same band of mRNA was observed during pre and post-natal periods.

Quantification of preproCCK mRNA: Densitometric scanning of the autoradiograms was used to quantitate mRNA. The suitability of the method was tested with an autoradiogram from a RNA blot of a gel of serial diluted poly(A)+RNA. Each lane of the gel on the autoradiograph was scanned and the weight of the peaks corresponding to the hand of preproCCK mRNA was measured. In the range of 1 to 15 μg of poly(A)+RNA, a linear relationship was observed between the density of the band on the autoradiograph and the amount of poly(A)+RNA applied to the gel.

Developmental regulation of preproCCK mRNA levels: Brain preproCCK mRNA was detectable at embryonic day 14. It increases progressively to reach maximum levels two weeks after birth, and tended to decrease slightly in young adult rats.

Developmental regulation of CCK-IR concentrations: CCK-IR was undetectable at embryonic day 14. Detectable levels were measured by embryonic day 20, but marked development was seen only after birth with a rapid increase between post-natal days 7 and 21, CCK-IR content per brain increasing ten folds during this period. By three weeks, the content reached approximately 90% of the adult levels.

Significance of Mental Health Research: The peptide CCK is contained in some dopaminergic neurons. Because dopamine has been continuously implicated in the pathogensis of schizophrenia, elucidating the biochemistry of dopaminergic neurons holds much promise for increasing our understanding of the biology of this syndrome.

Proposed Course of Project: Continuation of this project will focus on both the regional distribution of CCK-RNA in human brain and studies of possible alternation in schizophrenic brains.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02250-01 NPB

PERIOD COVERED

October 31, 1984 through September 30, 1985

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

Purification of Messenger RNAs Encoding for Neurotropic Factors in the Rat Brain

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

Anne-Marie Duchemin, M.D., Visiting Fellow, NPB, IRP, NIMH

Dr. Richard Jed Wyatt, Chief, Neuropsychiatry Branch, IRP, NIMH; Dr. Tan Than Quach, Laboratory of Preclinical Pharmacology, IRP, NIMH; Dr. Erminio Costa, Chief, Laboratory of Preclinical Pharmacology, IRP, NIMH

COOPERATING UNITS *(if any)*

Laboratory of Preclinical Pharmacology, IRP, NIMH

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.25	.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.)*

It has been shown that brain injury induces the appearance of neurotrophic activity at the lesion site. The neurotrophic activity can be assayed in vitro on sympathetic and parasympathic neurons in culture.

Because of the potential importance of this and other neurotrophic and neurite promoting factors for much of the work of the Neuropsychiatry Branch, we decided to determine if we could use techniques from molecular biology to get a better understanding of their function.

The first steps of the project have consisted in the development of the assays for testing for neurotrophic activity and their production. Different techniques were used for identification of lesioned brain-specific mRNAs. Messenger RNAs were prepared from the walls of the wound cavity of lesioned rat brains and from equivalent areas of controls. They were fractionnated by sucrose density gradient centrifugation. Each fraction was translated into proteins in Xenopus oocytes and the products tested for neurotrophic activity in the neuron cultures. The fraction of mRNA from lesioned brains responsible for the neurotrophic activity was used as a template for cDNA synthesis. The cDNA was hybridized with mRNA from controls to subtract the sequences common to the two preparations.

PHS 6040 (Rev. 1/84) GPO 904-917

Project Description:

Objectives: Nerve Growth Factor (NGF) is the only neurotrophic factor with an established physiological role. In the peripheral nervous system, however, the action of NGF is restricted to sympathetic and sensory neurons. During the last several years, the existence of other neurotrophic factors has been demonstrated. It has also been suggested that following injury of the central nervous system, neurite promoting and neurotrophic factors are made available to facilitate repair.

The purpose of this project was the purification of one of these neurotrophic factors which seem to be produced in the brain after injury. These factors could be useful for increasing the functional effects of brain tissue transplantations. One of the major obstacles to clinical applications of brain grafts is their limited efficacy. The availability of neurotrophic factors could enhance growth of the grafts.

Methods Employed: The first steps of the project were development of the assays for testing the neurotrophic activity and the production of neurotrophic factors.

A. Techniques for Assaying Neurotrophic Activity:
Culture of neurons provide convenient assays of trophic factors activity. The survival of the neurons in response to an added factor can be readily quantified and used as a measure of the amount of trophic activity present. Embryonic ganglia from the peripheral nervous system provide a high yield of neurons and offer a fairly homogenous neuronal population.

Cell Preparation:
Sympathetic and dorsal root ganglia from day 12 chick embryos were dissected and pooled. At the end of the collection step, the ganglia were dissociated by incubation in a 0.25% trypsin medium. The dissociated cells were resuspended in culture medium supplemented with fetal calf serum and counted under a phase contrast microscope. Then the cell-suspension, containing 50% neurons, was diluted for seeding.

Cell Culture:
Falcon microtiter plates were treated with polyornithine and polyornithine-binding neurite-promoting factor (PNPF) to provide a neurite-conducive substriatum to facilitate recognition and counting of the test neurons. PNPF was obtained from serum-free media conditioned over RN 22 schwannoma cultures. The plates were then filled with the trophic factor solution to be tested and the cell suspensed. The cultures were incubated at 37°C in 5% CO_2-air mixture. After 24 hours, the cultures were fixed in 2% glutaraldehyde medium, stained with blue trypan and analyzed for neuronal survival under a phase-contrast microscope.

All assays include negative control cultures, unsupported by exogenous trophic factors, and positive control cultures, supported by an excess of NGF.

B. Source of Neurotrophic Factors:
We used injured brains of rats as source of neurotrophic factors. New-born or young adult rats (Sprague-Dawley) were anesthesized and mechanical wounds were inflicted by aspiration of 1-10 mm^3 of the parietal cortex. The wound cavity was filled with gelfoam sponge. After sacrifice, gelfoam fragments and brain tissue adjacent to the wound were removed. Control tissue were removed from non-injured rats. Tissue samples and gelfoam fragments

were homogenized in culture medium and centrifuged. The supernatants were tested for neurotrophic activity on the cell culture experiments.

Findings: We found that, without any exogenous support, less than 10% of the cultured neurons from chick embryo dorsal root and sympathetic ganglia, survive after 24 hours culture. With NGF (300 u/ml), 35% of the neurons survive and show dendrite processes. The extracts from the walls of the brain wound of young adult rats, killed 10 days after lesion, elicite 50% of neuron survival with formation of a dense network of processes whereas the extracts from control brain elicite only 15% of neuron survival. The maximal neurotrophic activity from young adult rats was found between 7 and 10 days after lesion.

The fact that these neurotrophic factors are probably proteins, indicate that they can be isolated using classical protein purification methods.

We choose, however, to purify these factors by using the recombinant DNA techniques. After isolating the messenger RNA encoding for neurotropic proteins, we can translate these messengers into proteins in cell-free systems and test the activity of the synthesized proteins in the neuron culture model. On the other hand, recombinant DNA can be prepared from the isolated mRNA and cloned. Then, the primary structure of the protein can be deduced.

To identify the lesioned brain-specific mRNAs, we used the differential hybridization technique. Messenger RNAs were prepared from the walls of the wound cavity of lesioned rat brains and from equivalent areas of controls. They were fractinated by sucrose density gradient centrifugation. Each fraction was translated into proteins in frog oocytes and the products tested for neuronotrophic activity in the neuron cultures. The fraction of mRNAs from lesioned brains responsible for the neuronotrophic activity was used as a template for labeled cDNA synthesis by enzymatic conversion with reverse transcriptase. The cDNA was hybridized with mRNA from control brains to substract the sequences common to the two preparations.

The non-hybridized cDNA, containing sequences specific to lesioned brains will be used to identify complementary, lesion-specific mRNAs. This specific mRNAs will be translated into proteins in vivo and specific cDNA will be cloned.

Significance to Mental Health Research: We look forward to this project accruing data that may be applied to the understanding and treatment degenerative diseases of the CNS such as Schizophrenia and Alzheimer's.

Proposed Course of Project: We expect to analyze the structure of the proteins and sequence the correspondent given over the next reporting year. Also, antibodies will be developed against the protein to study its distribution in the brain.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 02251-01 NPB

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Distribution of Brain Somatostatin mRNA

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Anita Feenstra, Ph.D., Visiting Associate, NPB, IRP, NIMH

Dr. Jean Cadet, Medical Staff Fellow, NPB, IRP, NIMH; Dr. Richard Jed Wyatt, Chief, Neuropsychiatry Branch, IRP, NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH
Neuropsychiatry Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	1	

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.)*

We have initiated a study of the local distribution of somatostatin mRNA in brains of patients with Huntington's disease and schizophrenia. Brains of patients with Huntington's disease and schizophrenia and normal brains are being collected.

PHS 6040 (Rev. 1/84)

GPO 904-917

Project Description:

Objectives: Studies showed that in Huntington's disease somatostatin-like immunoreactivity is elevated in the basal ganglia and nucleus accumbens, in schizophrenia there is a significant elevation in the lateral thalamus. Our oubjectives are to study the local distribution of somatostatin on the transciptional level and establish if the mRNA distribution is comparable to that of the peptides.

Methods Employed: A c-DNA clone of human somatostatin-I was obtained from W.J. Rutter and amplified in our laboratory. Brain specimens are being collected, RNA, DNA and protein will be extracted. The somatostatin mRNA will be quantified by Northern Blots using the human C-DNA clone as a probe. The protein is measured by radioummunassay.

Findings: Data are now being collected. Analyses will be performed during the next reporting year.

Significance to Mental Health Research: In view of its wide distribution and important physiological effects, studies of brain somatostatin in various mental diseases are should provide usetul data. The availability of its C-DNA allows the study of the site and regulation ot its synthesis.

Proposed Course ot Project: Data collection will continue into the next reporting year. Preliminary findings should be reported in next year's Annual Report.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02252-01 NPB

PERIOD COVERED

October 1, 1984 through September 30, 1985

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

Behavioral Pharmacology and Toxicology

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

William J. Freed, Ph.D., Chief, Preclinical Neurosciences Section, NPB, IRP, NIMH

Dr. Renaud de Beaurepaire, Visiting Associate, NPB, IRP, NIMH; Dr. Jack A. Grebb, Clinical Research Associate, NPB, NIMH; Dr. Emad H. Ghoz, Faculty of Medicine, Cairo Univeristy, Cairo, Egypt; Dr. Saul Schwartz, Department of Neurosurgery, Naval Medical Center, Bethesda, Maryland

COOPERATING UNITS *(if any)*

Cairo University, Cairo, Egypt
Department of Neurosurgery, Naval Medical Center, Bethesda, Maryland

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Preclinical Neurosciences Section

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.25	.75	.50

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 on behavioral pharmacology and toxicology is derived from an ongoing interest in brain fucntion, from the presumption that schizophrenia is a disease of the brain and from a belief that behavioral studies can be windows on in vivo processes. The studies continued through this past reporting year have yielded a series of interesting results suggesting new directions for future research.

PHS 6040 (Rev. 1/84) GPO 904-917

Project Description:

Objectives: This research program is based on the presumption that schizophrenia is a disease of the brain, and is aimed at the development of animal models of brain dysfunction and developmental arrest induced by pharmacological agents, as well as the development of pharmacological agents to alleviate the behavioral manifestations of these manipulations. Although the ultimate overall objective is the development of agents for the treatment of schizophrenia and/or elucidation of the causes of schizophrenia this is a long-range goal and significant progress in this specific objective be extremely elusive. In the meantime, significant gains may be made in basic neuropharmacology and neurotoxicology, and some contributions to the neuropharmacology of other disorders such as epilepsy and Parkinson's disease may be made.

Methods Employed: Behavioral studies include observations of seizures, measurements of general locomotor activity, tests of maze-learning and operant conditioning, measurements of rotational behavior and measurements of feeding and drinking behavior. Animals will also be subject to the induction of brain lesion through the administration of drugs and neurotoxins as well as stereotaxic injection of neurotoxic substances. Histological studies are also performed for some experiments.

A. Behavioral Pharmacology of Phencyclidine

Objectives: This research program is aimed at the development of animal models for the measurement of behavioral responses to phencyclidine (PCP), employment of these models to assess the ability of various agents to antagonize the behavioral effects of PCP, and to characterize the behavioral pharmacology of PCP. The immediate purpose of the experiments is the development of pharmacological gents for the treatment of adverse reactions of PCP abuse. The more long-range goal is the development of agents for the treatment of schizophrenia and/or eludication of the causes of schizophrenia.

Methods Employed: Behavioral observations and testing for general locomotor activity, etc. following systemic or intracerebral administration of PCP.

Major Past Findings: Phencyclidine, or PCP, is considered to be one of the best pharmacological models of schizophrenia because of the wide range of psychological reactions to PCP abuse, including sensory disturbances (rather than vivid visual hallucinations as with LSD) as well as activation similar to that produced by amphetamine. In addition, violent and even prolonged psychotic reactions to PCP abuse are common. In initial studies a large series of pharmacological agents were screened for their ability to block PCP reactions in mice. Only a few agents, including phenothiazine neuroleptics, GABA agonists, and yohimbine and methysergide, were effective behavioral antagonists of PCP. Twenty-six other agents were ineffective, including some that are used clinically such as diazepam and haloperidol. In a subsequent study, a variety of neuroleptics were tested for their ability to antagonize PCP. Significant differences among neuroleptis were found, with the most effective agents being methiothepin and fluphenazine, as well as phenothiazines in general, while haloperidol, pimozide, molindone, and sulpiride were generally ineffective. A study of the genetics of PCP reactions in recombinant inbred strains of mice has also been performed, for the purpose of developing animal models of severe vs. mild PCP reactions in man, as well as to determine whether reactivity to PCP is determined, and unrelated to responsivity to amphetamine. BALB strain mice showed pronounced reactions to PCP, while C57 Bl/6 mice reacted much less markedly (about one-third as much locomotor stimulation). Most of the recombinant strains showed intermediate

reactions. The BALB strain mice may therefore provide a good model for severe PCP reaction in man.

New Findings: 1. Correlational analysis of the data from the effects of neuroleptics on PCP-induced stimulation suggested that the ability of neuroleptics to block PCP responses was related to both antiserotonergic and antidopaminergic properties of the drugs. This conclusion is further supported by the pharmacological properties of yohimbine and methysergide. We have therefore administered small dosages of cinnanserin and haloperidol to rats in combination. This combined treatment appears to be capable of blocking the activation produced by PCP.
2. A study by Dr. Grebb (reported separately) has found that several calcium channel inhibitors are potent antagonists of behavioral reactions to PCP in mice. The CCI's of the dihydropyridine class were particularly effective, while the piperazines, papveriness, and others were ineffective. Nifedipine was effective in blocking both PCP and amphetamine responses.
3. Attempts to produce prolonged or severe reactions to PCP in mice by chronic administration have thus far been unsuccessful.
4. Steps have been taken to undertake comparative studies of neuroleptics in the treatment of adverse reactions to PCP in patients admitted to Saint Elizabeths Hospital. This will provide both an application of the data obtained in the animal models and a means of validating the animal model used here for potential human applications.

Proposed Course of Project A: One outgrowth from Dr. Grebb's studies using the PCP animal model is the possibility of clinical trials of nifedipine in the treatment of schizophrenia. Additional animal drug trials are also planned as part of a continuing effort to develop PCP antagonists. Additionally, evaluation of pharmacological antagonism of PCP in BALB vs. C57Bl/6 mice is being considered. Finally, steps to develop an additional animal model, consisting of measurement of the effects of PCP on startle responses in rats or other rodents has been initiated.

B. Seizures and Amino Acids

The excitatory amino acids, glutamate and aspartate, and their decarboxylated inhibitory counterparts GABA and glycine are major and ubiquitous putative regulators of neuronal excitation and inhibition. Excitatory neurotransmitters potentially may be involved in neuropsychiatric disorders in two distinct ways: (1) Disturbances in amino acid neurotransmitter function may be involved directly in epilepsy, and possibly in schizophrenia. Schizophrenia is exacerbated by administration of amino acids such as methionine, and there is some evidence that the effects of methionine are due to metabolic conversion to the excitatory substance homocysteine. The disease homocystinuria is also accompanied by behavioral disturbances. (2) There is increasing evidence that injury to neurons which is caused by excess stimulation by excitatory amino acids, such as glutamate or kainic acid, is a major mechanism of CNS neurotoxicity, and has been hypothesized to be involved in Huntington's chorea as well as the neuronal damage consequent to ischemia. Thus there is a potential involvement of excitatory amino acid toxicity in schizophrenia and other neuropsychiatric disorder as well.

Methods Employed: These studies are conducted primarily by administration of drugs, either systemically or intracerebrally into the lateral ventricles, followed by observation and blind scoring of seizures. Seizures are induced by chemical agents, auditory stimulation, or electrical stimulation of the brain. Future studies also involve induction of seizures by administration of amino acids agonists, such as quisqualate, directly into the cerebral ventricles. Additional studies which are being planned involve administration of excitatory

substances to mice on neonatal day 1 followed by measurement of weight gain and histological assessment of neuronal damage.

Major Past Findings: A considerable body of literature in the past has shown that schizophrenia can be exacerbated by administration of the amino acid methionine. We obtained evidence from animal studies that some of the effects of methionine appeared to be due to accumulation of its metabolite homocysteine, rather than to increases in methylation reactions as was originally supposed. Evidence has also been obtained that homocysteine is a relatively specific agonist of the quisqualate-sensitive or "Type II" excitatory amino acid receptor site. Betaine, which is involved in the remethylation of homocysteine, has also been found to have anticonvulsant properties. Homocystinuria, a disorder involving excess accumulation of homocysteine and related amino acids in addition to mental retardation and seizures, has been reported by others to be successfully treated with betaine. This area is also of interest as induction of brain damage by excessive excitatory amino acids has recently been recognized as a major neurotoxicological process and could be involved in the genesis of developmentally-related brain abnormalities.

New Findings: 1. Although betaine is not metabolically active in the brain, its anticonvulsant effect is mediated by the brain. Betaine was about 1000-fold more potent in inhibiting seizures when administered directly into the brain as compared to peripherally. Betaine was also found to be capable of blocking metrazol and ECS-induced seizures, but does not antagonize audiogenic or kindled seizures. 2. Betaine, a derivative of glycine with three methyl groups, and its two metabolites, dimethylglycine and sarcosine, or monomethyl glycine were found to be similarly potent in blocking seizures and death induced by strychnine. This effectively rules out any metabolic effect of betaine in the antagonism of seizures. 3. An extensive structure activity study of glutamate diethyl ester (GDEE), the prototype antagonist of the quisqualate receptor, is underway and nearly complete. Information from this study may be useful in finding pharmacological antagonists of the effects of homocysteine and methionine. Incidentally, such drugs could also be of use in the treatment of certain types of seizures, such as those induced by alcohol withdrawal. One GDEE derivative, glutamate tert-butyl ester, was unexpectedly found to have pronounced convulsant properties.

Proposed Course of Project B: Steps are being taken to develop an animal model of developmental abnormalities induced by excessive excitatory amino acid receptor stimulation. The obesity of mice induced by postnatal administration of monosodium glutamate, using the protocols that have previously been developed may be an excellent model. Such a model can then be employed to test drugs and various agents for an ability to antagonize the toxic effects of excitatory amino acids.

Additional studies that may be performed include testing of additional agents, particularly acridines and chlorpromazine, for antagonism of seizures induced by homocysteine, development of a model for the induction of seizures by specific stimulation of each of the three major excitatory amino acid receptor sites, attempts to antagonize behavioral effects of methionine other than seizures, and studies of the effects of chronic administration of betaine and homocysteine. The startle response system, once available, will also provide an additional method for testing effects of these amino acids and antagonism of these effects.

C. Developmental Arrest

Developmental arrest consists of brief interference with development of the central nervous system, usually induced by the administration of short-acting antimitotic agents during critical periods of brain development. Such models can be employed to produce diffuse but

relatively restricted brain abnormalities, such as reductions in cortical thickness or volume of the striatum. Administration of the antimitotic agent methylazoxymethanol (MAM) to rats on the fifteenth day of gestation, for example, induces behavioral hyperactivity, putative deficits in learning, and hyperinnvervation of the cerebral cortex by catecholaminergic fibers. The potential relevance of this model to neuropsychiatric disorders has prompted us to conduct additional studies.

Major Past Findings: Developmental arrest induced by the antimitotic agent MAM serves as a model of abnormal development due to brief interference with CNS growth. When administered during growth of the cerebral cortex, the cerebral cortex does not fully develop, leading to a variety of behavioral abnormalities. This model may parallel some of the minor abnormalities of CNS structure recently reported in schizophrenia. One of the concomitants of this model is an excessively dense catecholaminergic innervation of the cerebral cortex.

We have previously measured a variety of behavioral indices in animals with developmental arrest induced by MAM. Essentially all of the observed abnormalities could be explained by the presence of hyperactivity in the animals which had been treated by MAM; for example, learning abnormalities were present only when the learning tasks required increased behavioral output for successful performance.

New Findings: Studies of maze-learning capacity of animals prenatally treated with MAM are underway and nearly completed. It is hoped that maze learning will provide a measure of learning that is relatively independent of levels of activity.

Proposed Course of Project C: Measurements of response of MAM-treated rats to amphetamine and apomorphine are planned. As information about CNS pathology and brain atrophy in schizophrenia continues to develop, attempts to produce a developmental arrest model of schizophrenia may receive additional impetus.

D. Calcitonin

Calcitonin, a peptide hormone secreted by the C-cells in the thyroid, is primarily involved in the regulation of peripheral calcium metabolism. Our group has found that calcitonin is also, however, a very potent inhibitor of eating behavior, and this effect is mediated directly by the brain. Calcitonin has subsequently been reported to inhibit amphetamine-induced stimulatory effects. Even though calcitonin is not produced by the brain it may be an important peripherally-derived hormonal regulator of behavioral processes through actions on the central nervous system.

Methods Employed: Animals receive chronic cannula implants into various brain nuclei or into the lateral ventricle using standardized procedures. Calcitonin is then administered through the cannulae in small amounts, and eating behavior, activity, and other behavioral responses are measured. In some experiments, the animals also receive systemic injections of d-amphetamine or calcitonin.

Major Past Findings: We have previously found that calcitonin is an extremely potent inhibitor of eating behavior in animals, and this effect has been found to be mediated via the CNS. Others have reported that calcitonin is capable of inhibiting amphetamine-induced behavioral stimulation. There is some evidence that calcitonin is a hormone that serves to modulate calcium uptake by certain CNS neurons.

New Findings: 1. We have attempted to specifically localize the site of action of calcitonin within the CNS by measurement of behavioral responses to calcitonin following injection through chronically implanted 33-guage cannulae. Responses to calcitonin were obtained from several hypothalamic nuclei, especially the paraventricular nucleus, the perifornical area, the supraoptic nucleus, ventromedial nucleus, and nucleus reuniens. Responses were also obtained from the vertical limit of the diagonal band and from the nucleus accumbens. 2. A second study involved measurements of the responses to calcitonin as a function of age. The peripheral effects of calcitonin are known to diminish with age. We found that the CNS effects did not decrease with age, suggesting that the CNS effects and peripheral effects are independent.

Proposed Course of Project D: Attempts to determine whether inhibition of eating behavior by calcitonin and inhibition of responses to amphetamine are localized in the same brain regions are underway and nearly complete. Further attempts to determine whether the behavioral effects of stimulant and psychotomimetic agents (responses to PCP, apomorphine-induced rotational behavior) can be inhibited by calcitonin are also planned (see "phencyclidine" section).

Significance to Mental Health Research: If it is accepted that schizophrenia is the result of some brain injury or insult, the possibilities for the genesis of the abnormality are relatively limited. Some of these possibilities include the action of neurotoxins, psychotogens such as PCP, or developmental arrest. This program attempts to investigate some of these possibilities in animal models. In the realm of neurotoxicology, two of the most promising models are the excitotoxic model, i.e., the induction neuronal death through the actions of excitatory amino acids or their analogues, and the particular sensitivity of the dopamine systems of the brain to neurotoxins, such as manganese the recently discovered agent MPTP. Attempts to develop models for further characterizing these models of neurotoxicity are underway. As information about the pathology of the brain in schizophrenia continues to develop, it is anticipated that the various models can be refined and further developed.

Proposed Course of Project: This project is expected to continue indefinitely, as an interactive process with clinical sections of the branch.

Publications:

Freed, W.J., Bing, L.A. and Wyatt, R.J.: Effects of neuroleptics on phencyclidine (PCP)-induced locomotion stimulation in mice. Neuropharmacology 23:175-181, 1984.

Freed, W.J., Bing, L.A., Anderson, A.E. and Wyatt, R.J.: Calcitonin as an anorectic agent. In Shah, N.S. and Donald, A.G. (Eds.): Psychoneuroendocrine Dysfunction in Psychiatric and Neurological Illness: Influence of Psychopharmacological Agents. New York, Plenum Publishing Corporation, 1984, pp. 83-109.

Freed, W.J.: Selective inhibition of homocysteine-induced seizures by glutamic acid diethyl ester and other glutamate esters. Epilepsia 26:30-36, 1985.

Freed, W.J.: Prevention of strychnine-induced seizures and death by the N-methylated glycine derivative betaine, dimethylglycine and sarcosine. Pharmacol. Biochem. Behav. 22:641-643, 1985.

Ghoz, E.H. and Freed, W.J.: Effects of betaine on seizures in the rat. Pharmacol. Biochem. Behav. 22:635-640, 1985.

Freed, W.J., Ghoz, E.H. and Crump, S.: Convulsant properties of L-glutamic acid di-tert butyl ester. Neurobehav. Toxicol. Teratol., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02253-01 NPB

PERIOD COVERED

October 1, 1984 through September, 30, 1985

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

Brain Tissue Transplantation

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

William J. Freed, Ph.D., Chief, Preclinical Neurosciences Section, NPB, IRP, NIMH

Dr. Urmi Patel-Vaidya, Staff Fellow, NPB, IRP, NIMH; Dr. Herbert Geller, Rutgers University, New Brunswick, New Jersey; Dr. Jeff Laskin of Rutgers University, New Brunswick, New Jersey; Dr. Jeffrey Greenberg, Neurosurgeon Resident, Georgetown University; Dr. Richard Jed Wyatt, Chief, Neuropsychiatry Branch, IRP, NIMH; Dr. Barry Hoffer, Department of Pharmacology, University of Colorado, Denver, Colorado

COOPERATING UNITS *(if any)*

Rutgers University, New Jersey; Georgetown University; Karolinska Institute, Stockholm, Sweden; Johns Hopkins University; VA Medical Center, Washington, D.C.; University of Colorado, Denver, Colorado

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Preclinical Neurosciences Section

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.75	1.75	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.)*

These studies of brain tissue transplantation in non-primate animals attempt (1) to develop the techniques of brain tissue transplantation so that it may be applied clinically to Parkinson's disease. (2) To develop brain tissue transplantation techniques for eventual application to other disorders, such as schizophrenia or Alzheimer's disease if and when these disorders become well enough understood to permit such applications, and (3) to employ brain tissue transplantation as a technique to elucidate factors which control the development and plasticity of the brain, particularly the nigrostriatal dopamine system. During the past reporting year important progress has been made toward achieving these goals.

PHS 6040 (Rev. 1/84) GPO 904-917

Collaborators:

Dr. Lars Olson, Dept. of Histology, Karolinska Institute, Stockholm, Sweden
Dr. Ingrid Stromberg, Dept. of Histology, Karolinska Institute, Stockholm, Sweden
Dr. John M. Morihisa, Staff Psychiatrist, NPB, IRP, NIMH
Dr. Debra Niehoff, Department of Neuroscience, Johns Hopkins University
Dr. M. Kuhar, Department of Neuroscience, Johns Hopkins University
Dr. M.R. Wells, VA Medical Center, Washington, D.C.
Dr. Luis de Medinaceli, Visiting Scientist, NPB, IRP, NIMH

Project Description:

Objective: The major overall objective of this program is to develop brain tissue transplantation as a technique for the repair of localized damage to the central nervous system. There are three more specific objectives: (1) to develop the techniques of brain tissue transplantation so that it may be applied clinically to Parkinson's disease. (2) to develop brain tissue transplantation techniques for eventual application to other disorders, such as schizophrenia or Alzheimers disease if and when these disorders become well enough understood to permit such applications, and (3) to employ brain tissue transplantation as a technique to elucidate factors which control the development and plasticity of the brain, particularly the nigrostriatal dopamine system.

Methods Employed: These studies involve surgical, behavioral and histological-histochemical procedures in animal subjects.

Major Past Findings: Grafts of embryonic substantia nigra or young adult adrenal medulla have been shown to decrease rotational behavior consequent to unilateral lesions of the substantia nigra (SN). The SN grafts produce dopamine and reinnervate the host caudate-putamen, and decrease spiroperidol binding in the striatum concomitant with their behavioral effects. The adrenal medulla grafts also produce dopamine, but do not reinnervate the host brain, apparently exerting their behavioral effects simply through secretion of catecholamines followed by diffusion into the host brain tissue. Although these intracerebral grafts survive indefinitely across major histocompatibility typings, it has been found to be possible to induce rejection through peripheral sensitization of the host animals. It should be emphasized that the behavioral effects of both adrenal medulla grafts and SN grafts are relatively limited in magnitude: In general, the SN grafts appear to be limited because of a limited reinnervation of the host brain, while the adrenal medulla grafts are limited due to limited survival of the grafted cells.

New Findings: Attempts have been concentrated on improving survival rate for grafted adrenal chromaffin cells, developing alternative implantation techniques, and characterizing the factors that are involved in limiting or facilitating the reinnervation of the host brain SN grafts.

1. Effects of Ganglioside GM1, estrogen, and haloperidol on reinnervation of the host brain by SN grafts. All three of these substances have been tested for their effects on SN grafts, and all three have been found to be ineffective.

2. Implants of catecholamine-containing tumor cell lines: We have investigated the possibility of using catecholamine-containing tumor cells (PC12 cells and B16c neuro-melanoma) as sources of tissue for transplantation into the CNS. Results are generally disappointing. The numbers of PC12 cells that survive indefinitely are fairly small. B16c cells survive indefinitely in large numbers, and actually increase in number after implantation. In mouse recipients, these cells grow uncontrollably, as a tumor, and the animals die within two to four weeks. In rat recipients, growth is limited, but numbers of cells nonetheless increase several-fold after implantation. Unfortunately, however, the B16c cells produce catecholamines for only about two weeks after implantation after which they continue to produce melanin but no catecholamines.

3. Dissociated adrenal chromaffin cell implants: We have assessed the survival and growth of adrenal chromaffin cells when implanted into the parenchyma of the rat striatum. Although some cells survive, their numbers and apparent catecholamine production does not appear to be sufficient to produce substantial behavioral effects. Interestingly, implanted chromaffin cells form processes, or cytoplasmic extensions, extremely rapidly and frequently within one hour after implantation into the host brain. The rapidity of process formation

suggests possibly interesting trophic interations between the striatum and adrenal chromaffin cells.

4. Implants of solid pieces of adrenal medulla into striatal parenchyma: We have also assessed the survival and behavioral effects of solid pieces of adrenal chromaffin tissue implanted into the striatal parenchyma, in part because this procedure has been employed for human clinical trials in Sweden. Numbers of cells surviving permanently were again fairly small, less than 1000 per recipient animal. These small grafts were, however, intensely fluorescent when processed for catecholamine fluorescence, suggesting the production of fairly substantial amount of catecholamines per surviving cell. Adrenal medulla implants from both young and aging donors were examined. The grafts obtained from young donors tended to slightly decrease rotational behavior and deficits in sensorimotor performance. Grafts obtained from aging donors also slightly alleviated sensorimotor deficits but had no effect on rotational behavior. This study clearly suggests that obtaining increased survival of intraparenchymal adrenal medulla grafts is the key to useful grafts of this type.

5. Trophic effects on intraparenchymal adrenal medulla grafts: A major effort during the next year will be directed at assessing trophic effects and implantation techniques for intraparenchymal grafts of adrenal medulla. Studies performed thus far include evaluation of the effects of co-implantation of adrenal medulla with tissues containing corticosteroids (adrenal cortex), nerve growth factor (NGF) (mouse submaxillary gland) or other unidentified trophic substances (rat iris). These studies have been partially completed but the data are not yet decoded. Additional studies planned or underway include evaluation of effects of prior lesions of the implantation site, assessment of graft survival in inbred rat strains (to rule out the possibility of partial rejection of the grafts), and local administration of trophic substances such as NGF through osmotic minipumps.

6. Trophic effects of cortical lesions and striatal lesions on substantia nigra grafts: These studies are intended to exploit the possibility of secretion of trophic substances by damaged brain tissue to enhance the penetration of dopamine-containing fibers from SN grafts into the host brain tissue. In an initial experiment, cortical lesions were found to increase the growth of fibers from grafts into the host brain, but only in the most dorsal part of the striatum close to the lesioned brain area. A replication of the initial experiment is now nearly complete. A study of the effects of kainic acid lesions of the striatum on reinnervation of the striatum is also underway. An effort to automate or partially automate the assessment of fiber ingrowth has been initiated.

7. Development of a cat model: In order to have available another model for development of brain grafting for application to higher-order species, we have taken steps to develop brain tissue transplantation procedures for cats. We have developed a number of behavioral testing procedures and have developed procedures for producing complete unilateral lesions of the substantia nigra. No grafts have yet been implanted in cat subjects.

8. Application to other models: We have conducted very limited studies of brain tissue transplantation in two other models, involving cortical injury and blindness induced by enucleation, to further investigate the generality of functional effects of brain grafting. The study of cortical grafts was essentially a replication of a previous study in which cortical grafts were found to decrease some of the behavioral deficits induced by lesions of the medial frontal cortex. Our study partially confirmed this initial report. Studies of the possibility of functional effects of retinal grafts in blind, enucleated rats have been undertaken. No data are yet available are yet available, although all of the necessary methods have been developed.

Significance to Mental Health Research: These studies may lead to the development of brain tissue transplantation as a therapeutic procedure for Parkinson's disease and eventually for other disorders. In addition, brain tissue transplantation is a valuable technique for the investigation of trophic functions in the brain. For example, the putative finding that brain injury has a trophic effect on dopamine-containing neurites is of potential importance.

for the understanding of the developmental and trophic influences on the brain dopaminergic system and its possible dysfuntion in schizophrenia. Investigation of trophic functions and their possible absence is of particular importance for diseases such as schizophrenia, which may involve relatively subtle forms of neuronal dysfunction rather than readily detectable brain atrophy or neuronal degeneration.

Proposed Course of Project: The investigation of brain tissue transplantation as a therapeutic technique is expected to continue until a reasonably effective procedure which can be applied to Parkinson's disease is developed. Subsequently, grafting will be studied primarily as a means of assessing trophic control of development and function of the brain dopaminergic systems. Studies of possible application of brain tissue transplantation to other disorders will also be continued in particular as developments in other fields elucidate possible applications.

Publications:

Mackay-Sim, A. and Patel, U.: Regional differences in cell density and neurogenesis in the olfactory epithelium of the salamander, Ambystoma tigrinum. Exp. Brain Res. 57:99-106, 1984.

Patel-Vaidya, U., Wells, M.R. and Freed, W.J.: Survival of dissociated adrenal chromaffin cells of rat and monkey transplanted into rat brain. Cell Tissue Res. 240(2):281-285, 1985.

Freed, W.J., Olson, L., Ko, G.N., Morihisa, J.M., Niehoff, D., Stromberg, I., Kuhar, M., Hoffer, B.J. and Wyatt, R.J.: Intraventricular substantia nigra and adrenal medulla grafts: Mechanisms of action and ^{3}H-spiroperidol autoradiography. In Bjorklund, A. (Ed): Transplantation in the Mammalian CNS. Amsterdam, Elsevier Science Publishers, 1985, pp. 471-489.

Freed, W.J.: Transplantation of tissues to the cerebral ventricles: Methodological details and rate of grafts survival. In Bjorklund, A. (Ed.): Transplantation in the Mammalian CNS. Amsterdam, Elsevier Science Publishers, Amsterdam, 1985, pp. 31-40.

Freed, W.J., Cannon-Spoor, H.E. and Krauthamer, E.: Factors influencing the efficacy of adrenal medulla and embryonic substantia nigra grafts. In Bjorklund, A. (Ed.): Transplantation in the Mammalian CNS. Amsterdam, Elsevier Science Publishers, 1985, pp. 491-504.

Freed, W.J.: Ganglioside GM1 does not stimulate reinnervation of the striatum by substantia nigra grafts. Brain Res. Bull. 14:91-95, 1985.

Freed, W.J., de Medinaceli, L. and Wyatt, R.J.: Promoting functional plasticity in the damaged nervous system. Science 227:1544-1552, 1985.

Patel-Vaidya, U., Wells, M.R. and Freed, W.J.: Survival of dissociated rat and monkey adrenal chromaffin cells transplanted into rat brain. Cell Tissue Res. 240:281-285, 1985.

Wuerthele, S.M., Olson, L., Freed, W.J., Wyatt, R.J. and Hoffer, B.J.: Electrophysiology of substantia nigra transplants. In Usdin, E., Carlsson, A., Dahlstrom, A. and Engle, J. (Eds.): Neurology and Neurobiology, Volume 8B, Catecholamines, Part B: Neuropharmacology and Central Nervous System--Theoretical Aspects. New York, Alan R. Liss, Inc., 1985, pp. 333-341.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02254-01 NPB

PERIOD COVERED

October 1, 1984 through September 30, 1985

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

Calcium Channel Inhibitors: Interactions with Dopaminergic Systems - Animal Studies

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

Jack A. Grebb, M.D., Medical Staff Associate, NPB, NIMH

Dr. Richard C. Shelton, Medical Staff Associate, NPB, NIMH; Dr. Jean L. Cadet, Medical Staff Associate, NPB, NIMH; Dr. William J. Freed, Chief, Preclinical Neurosciences Section, NPB, NIMH; Dr. Gabriele Panza, Visiting Associate, Section on Biochemical Pharmacology, NHLBI, NIMH; Dr. Ingeborg Hanbauer, Section on Biochemical Pharmacology, NHLBI; NIMH

COOPERATING UNITS *(if any)*

Section on Biochemical Pharmacology, NHLBI

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.75	.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.)*

Calcium channel inhibitors (CCI's) and calcium channel activators (CCA's) are thought to affect calcium flux through membrane bound channels as their major site of action. The literature already contains reports of CCI's inhibiting PCP and amphetamine stimulation in animals. There are four major subclasses of CCI's, and each appears to have different biochemical and behavioral properties. Our animal studies have addressed the hetereogeneity of this class of drugs, and have been designed to test multiple CCI's in single animal behavior models. To date findings show that nifedipine-like CCI's block PCP stimulation, nifedipine or flunarizine blocks amphetamine-induced stimulation, co-administration of diltiazem or verapamil (but not nifedipine) with chronic haloperidol treatment inhibits the development of neuroleptic-induced apomorphine supersensitivity, but not amphetamine supersensitivity. Additional studies have suggested a complex interaction between dopamine receptor function and calcium channels.

PHS 6040 (Rev. 1/84) GPO 904-917

Project Description:

Objectives: Calcium channel inhibitors (CCI's) may be important new pharmacologic agents for neuropsychiatrists. Existing studies have suggested that the CCI's may affect dopaminergic systems, and be of clinical relevance to patients with schizophrenia or movement disorders. The CCI class of drugs is actually made up of at least four very different subclasses - papveriness, benzothiazepines, dihydropyridines and piperazines -which seem to have varying biochemical properties. The major objective for our animal research was to clarify which of the CCI subclasses had what effects on dopaminergic systems. In addition to the above areas, we have pilop studies in animals investigating the efects of CCI's on memory, and also into the potential role of a CCA induced seizure model in animals.

Methods: 1. Differential EFfects of CCI's on PCP-Induced stimulation in Mice. We pretreated (IP) mice with acute injections of 17 different CCI's and used activity tables to measure the effects on PCP-induced stimulation. 2. Differential Effects of CCI's on amphetamine-induced stimulation in Mice. The design was the same as for #1, except amphetamine was used instead of PCP. 3. Differential Effects of CCI's on Neuroleptic-Induced Behavioral Supersensitivity in Mice. We co-administered three different CCI's with haloperidol for one month to mice and then tested the mice for apomorphine-and amphetamine-induced behavioral stimulatin. The mice were then sacrificed and their brains were analyzed for regional ^{3}H-nitrendipine bindings. 4. Differential Effects of CCI's on Denervation Supersensitivity. As an additional model of dopaminergic supersensitivity we are chronically administering CCI's to rats that have had unilateal 6-OH-DA nigrostriatal lesions. We will assess these rats for the development of apomorphine-induced turning behavior. We will also sacrifice these rats and analyze their brains for dopamine receptor bindings. 5. Pilot studies of CCI's (a) Mice that have chronically received CCI's will be tested in a memory paradigm involving cage habituation. (b) Mice and rats develop seizures when given very small amounts of CCA's either peripherally or centrally. We are constructing a dose response curve for this effect, and will be using this model as a screening test for currently available anticonvulsants.

Major Past Findings: Our only finding completed before October 1, 1984 was that nifedipine-like drugs inhibited PCP-induced behavioral stimulation in mice whereas other classes of CCI's did not.

New Findings: 1. Nifedipine and flunarizine (but no other CCI's) inhibit amphetamine-induced behavioral stimulation in mice. 2. Co-administration of diltiazem or verapamil (but not nifedipine) with chronic haloperidol treatment inhibits the development of neuroleptic-induced apomorphine supersensitivity, but not amphetamine supersensitivity. 3. Chronic administration of nitrendipine or verapamil (but not diltiazem causes a decrease in ^{3}H-nitrendipine binding sites in brains in mice. 4. CCA's induce seizures in rodents and this is a central phenomenon. The dose required for this affect is very small.

Significance to Mental Health Research: CCI's are already in clinical use for the treatment of cardiac conditions and appear to have a favorable profile regarding side effects. If these drugs were found to be of benefit to human neuropsychiatric patients they would provide a valuable addition to the pharmacologic tools. Regardless of their eventual clinical use, the CCI's and CCA's are valuable and reasonably specific probes into the functioning of cells. By affecting membrane function of calcium channels we may be able to learn more about the function of other receptors as well as phosphorylation mechanisms.

Proposed Course of Project: The projects listed in "Methods" will be completed by June 22, 1985, and will be submitted both to scientific meetings and for publication. Drs. Grebb and

Shelton will be continuing CCI-related research at New York University and Vanderbilt University, respectively, and Dr. Cadet may continue CCI-related research at NPB.

Publications:

Panza, G., Grebb, J.A., Shelton, R.C. and Hanbauer, I.: Nitredipine-sensitive Ca^{2+} channels in mouse brain: Evidence for down-regulation of recognition sites after long-term treatment wtih nifedipine or verapamil. Fed. Proc. 44(4):884, 1985.

Grebb, J.A., Ellsworth, K.A. and Freed, W.J.: Differences between calcium channel inhibitors in their effects on phencyclidine-induced behavioral stimulation in mice. Pharmacol. Biochem. Behav., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 02255-01 NPB

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Calcium Channel Inhibitors: Interactions with Dopaminergic Systems - Human Studies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Jack A. Grebb, M.D., Medical Staff Associate, NPB, NIMH

Dr. Richard C. Shelton, Medical Staff Associate, NPB, NIMH; Dr. Llewellyn Bigelow, Director of WAW Division, Saint Elizabeths Hospital; Dr. Gabriele Panza, Visiting Associate, Section on Biochemical Pharmacology, NHLBI; Dr. Ingeborg Hanbauer, Section on Biochemical Pharmacology, NHLBI; Dr. Daniel R. Weinberger, Chief, Section on Clinical Neuropsychiatry, NPB, IRP, NIMH; Dr. Richard Jed Wyatt, Chief, Neuropsychiatry Branch, IRP, NIMH

COOPERATING UNITS *(if any)*
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LAB/BRANCH
Neuropsychiatry Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.25	.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.)*

Calcium channel inhibitors (CCI's) and calcium channel activators (CCA's) are thought to affect calcium flux through membrane bound channels as their major site of action. There are reports of clinical trials involving over 150 patients suggesting that CCI's also have beneficial effects in neuropsychiatric disorders. There are four major subclasses of CCI's, and each appears to have different biochemical and behavioral properties. Additional studies have suggested a complex interaction between dopamine receptor function and calcium channels. We are currently investigating verapamil and nifedipine as treatments for schizophrenia and tardive dyskinesia. Patients on these protocols are tested with regional cerebral blood flow and computer analyzed brain electrical activity mapping. The platelets of these patients are also studied for ^{3}H-nitrenidipine and ^{3}H-yohimbine binding, as well as for differential patterns of protein phosphorylation.

PHS 6040 (Rev. 1/84) GPO 904-917

Project Description:

Objectives: Calcium channel inhibitors (CCI's) may be important new pharmacologic agents for neuropsychiatrists. Existing studies have suggested that the CCI's may affect dopaminergic systems, and be of clinical relevance to patients with schizophrenia or movement disorders. The CCI class of drugs is actually made up of at least four very different subclasses - papveriness, benzothiazepines, dihydropyridines and piperazines - which seem to have varying biochemical properties. Our clinical work has specifically asked whether verapimil or nifedipine might be beneficial for patients with schizophrenia or tardive dyskinesia. In addition to behavioral ratings, these patients are being followed with other biologic measures to assess any other correlates of CCI treatment.

Methods: Clinical trials of verapamil and nifedipine in schizophrenia and tardive dyskinesia. In patients diagnosed by DSM-III as having schizophrenia or in patients with tardive dyskinesia we are conducting double-blind trials of two CCI's. The patients are being followed with BPRS. In addition to these behavioral ratings, other biologic measures are being made - regional cerebral blood flow, BEAM, platelet analysis for ^{3}H-nitrendipine bindings, ^{3}H-hoyimbine binding and differential phosphorylation patterns.

Major Past Findings: Our only finding completed before October 1, 1984 was that nifedipine-like drugs inhibited PCP-induced behavioral stimulation in mice whereas other classes of CCI's did not.

New Findings: Neither beneficial nor deleterious effects on psychological state have been demonstrated thus far.

Significance to Mental Health Research: CCI's are already in clinical use for the treatment of cardiac conditions and appear to have a favorable profile regarding side effects. If these drugs were found to be of benefit to human neuropsychiatric patients they would provide a valuable addition to the pharmacologic tools. Regardless of their eventual clinical use, the CCI's and CCA's are valuable and reasonably specific probes into the functioning of cells. By affecting membrane function of calcium channels we may be able to learn more about the function of other receptors as well as phosphorylation mechanisms.

Proposed Course of Project: The projects will be completed by June 22, 1985, and will be submitted both to scientific meetings and for publication. Drs. Grebb and Shelton will be continuing CCI-related research at New York University and Vanderbilt University, respectively.

Publications:

Grebb, J.A. and Reus, V.I.: Neurobehavioral chemistry and physiology. In Lange, G.H. (Ed.): Review of General Psychiatry, 1984.

Grebb, J.A., Weinberger, D.R. and Wyatt, R.J.: Schizophrenia -- A neuropsychiatric approach. In Asbury, A.K., McKhan, G.M. and McDonald, W.I. (Eds.): Diseases of the Nervous System. Philadelphia, W.B. Saunders and William Heinemann, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02256-01 NPB

PERIOD COVERED

October 1, 1984 through September 30, 1985

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

Defect Symptoms in Schizophrenia: Their Measurement, Correlates and Treatment

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

Andrei-C. Jaeger, M.D., Staff Psychiatrist, NPB, IRP, NIMH

Dr. Darrell G. Kirch, Staff Psychiatrist, NPB, IRP, NIMH; Dr. Joel E. Kleinman, Chief, Section on Clinical Brain Studies, NPB, IRP, NIMH; Dr. Llewellyn B. Bigelow, Director of WAW Division, NIMH; Dr. Craig N. Karson, Acting Chief, Section on Clinical Brain Studies, NPB, IRP, NIMH; Dr. Richard Jed Wyatt, Chief, Neuropsychiatry Branch, IRP, NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Clinical Brain Studies Section

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.20	1.20	

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.)*

Renewed interest in the role of negative symptoms in "defect state" schizophrenia encouraged us to develop a "Negative Symptom Rating Scale (NSRS)" to more efficiently measure this syndrome.

Negative symptoms together with poor premorbid history, cognitive impairments, inadequate response to neuroleptic treatment and enlarged cerebral ventricles have been clustered into the Category of Type II schizophrenia. The relationships among negative symptoms and the other elements of defect state schizophrenia are the object of an ongoing study. The main goal of this study is to identify more homogeneous subgroups of patients with schizophrenia and the development of specific modalities of treatment for these groups.

PHS 6040 (Rev. 1/84)

GPO 904-917

Project Description:

Objectives: Previous speculations about the defect state symptoms suggested that negative symptoms are trait dependent, and probably represent the expression of structural damage in the brain of schizophrenic patients. We are attempting to elucidate the interrelationships among the elements that constitute the putative type II syndrome of schizophrenia, to investigate its neuroendocrine concommitants and to explore new pharmacological treatment modalities targeted more specifically at this neuroleptic-refractory syndrome.

Methods Employed: The Negative Symptoms Rating Scale (NSRS) is a seven point (0-6) scale with one point of absence, consisting of 10 items, conceptually subgrouped in four subscales, assessing thought process, cognition, volition and affect. A semi-structured 15-minute interview using the items as the guideline is employed by a trained rater.

The population consists of patinets diagnosed according to DSM III criteria for schizophrenic admitted at WAW Building, Saint Elizabeths Hospital, Washington, D.C. who volunteer to participate in clinical research studies. The patients' answers, appearance and behavior are rated on the NSRS as they go through drug protocols and drug-free periods. Material available for evaluating each patient include comprehensive psychiatric and medical history, ventricular brain ratio, drug response data as measured by the Brief Psychiatric Rating Scale (BPRS), and cognitive testing. All the data are collected, scored, and analysed independent of the biochemical data.

Blood samples from each patient are obtained after four weeks placebo and four weeks of fixed dose neuroleptic treatment (0.4 mg/kg body weight haloperidol a day divided in a twice a day regimen). The samples are drawn through an indwelling intravenous catheter with heparine lock at 30 minute intervals starting at 8:00 a.m. until 10:30 a.m. These samples are assayed for prolactin, growth hormone, renin, angiotensin, aldosterone, vasopressin and LH-FSH.

Major Past Findings: NSRS is convenient, reliable, easy to use and sensitive against the most frequently used negative symptoms scales or subscales. Further characterization of the scale including a factor analysis and a correlation matrix is in progress.

New Findings: In a preliminary study of 27 patients with DSM III schizophrenia and six patients with other DSM III psychiatric diagnoses (including bipolar disorder, schizoaffective, manic, paranoia and personality disorders) we found that the first group had much higher scores on NSRS than the latter ($p < 0.005$, $U=11.5$, two-tailed Mann-Whitney U test for two independent groups).

A recent preliminary controlled trial with a vasopressin analogue (DDAVP) reduced negative symptoms as measured by NSRS by 16% in a group of 10 schizophrenic patients. The greatest reduction in negative symptoms was noted in the two patients who did not receive neuroleptics during the trial.

Significance to Mental Health Research: Defect symptoms in schizophrenia are refractory to traditional antipsychotic treatment. This may suggest that at least in a large group of patients with schizophrenia cerebral pathology may involve a structural or functional substrate which includes in addition to monoaminergic transmission other modulatory and/or regulatory pathways. These studies lead to the possibility of identifying more precisely

these structures and their role in behavior and in turn this may lead to more efficatious treatment modalities of this socially paralyzing syndrome.

Proposed Course of Project: We plan to extend our study and collaboration in this area with the Special Treatment Unit of Creedmoor Psychiatric Center, Queens Village, New York.

Publications:

Iager, A-C., Kirch, D.G. and Wyatt, R.J.: A Negative Symptom Rating Scale. Psychiatry Res. (in press).

Iager, A-C., Kirch, D.G., Bigelow, L.B. and Karson, C.N.: Vasopressin treatment of schizophrenia. Am. J. Psychiatry (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02257-01 NPB

PERIOD COVERED

October 1, 1984 through September 30, 1985

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

Biochemical and Neuroradiologic Abnormalities in Tardive Dyskinesia

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

Dilip V. Jeste, M.D., Medical Officer, NPB, IRP, NIMH

Dr. Charles A. Kafumann, Senior Staff Fellow, NPB, IRP, NIMH; Dr. James Lohr, Medical Staff Fellow, NPB, IRP, NIMH; Dr. Alex Wisniewski, Medical Staff Fellow, NPB, IRP, NIMH; Dr. John Cadet, Medical Staff Fellow, NPB, IRP, NIMH; Dr. Richard Jed Wyatt, Chief, Neuropsychiatry Branch, NIMH, IRP; Dr. Markku Linnoila, NIAAA, NIH; Dr. Marian Kafka, Research Physiologist, NSB, NIMH; Dr. D.R. Doongaji, K.E.M. Hospital, Bombay, India

COOPERATING UNITS *(if any)*

NIAAA, NSB, NIMH; K.E.M. Hospital, Bombay, India

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Section on Aging

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.50	.50	

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 measured plasma dopamine-beta-hydroxylase (DBH) activity; norepinephrine, dopamine and their metabolites in cerebrospinal fluid (CSF); and indices of brain atrophy on CT scans in patients with and without tardive dyskinesia (TD). Patients with TD had greater plasma DBH activity and higher CSF-norepinephrine than non-TD patients. The two groups were similar in CSF-dopamine and its metabolites. Among patients with low plasma-DBH activity, those with TD had greater ventricular enlargement and higher bifrontal-bicaudate ratio, suggesting greater subcortical atrophy as compared with non-TD patients. These findings suggest that TD may be a heterogeneous syndrome, with subgroups characterized by noradrenergic hyperactivity or subcortical atrophy.

PHS 6040 (Rev. 1/84) GPO 904-917

Project Description:

Objectives: Tardive dyskinesia (TD), an extrapyramidal syndrome occurring late in the course of neuroleptic treatment has been the focus of increasing concern, yet its pathophysiology remains unknown. The most widely held theory postulates that striatal postsynaptic dopamine (DA) receptor supersensitivity results from prolonged neuroleptic exposure and underlies the abnormal movements. While popular, this theory does not provide an adequate model for the development of TD.

Abnormalities in another catecholamine, norepinephrine (NE), have been described in other movement disorders including Huntington's disease, Parkinson's disease, and autosomal dominant torsion dystonia. Moreover, pharmacologic data, ordinarily taken as evidence of DA supersensitivity in TD, are equally consistent with noradrenergic (NA) abnormalities.

While plausible, NA abnormalities in TD have received relatively little attention and reports have often been conflicting. These conflicting data might reflect, in part, biological heterogeneity in TD, which possibly includes biochemical subtypes.

Similar controversy exists for the role of structural brain pathology in the development of TD: some authors have described CT abnormalities in patients with TD; others have not. Once again, this might be consistent with biological heterogeneity in the disorder. Some of the controversy surrounding neurochemical and neuropathological abnormalities in TD might also be related to studying these parameters in isolation: we found no report which simultaneously measured noradrenergic and neuroradiologic indices in the same population.

The current work was undertaken to systematically assess catecholaminergic activity and neuroradiologic abnormalities in the same patients with and without TD. Biochemical measures included plasma DBH activity and (in patients consenting to lumbar puncture) CSF NE, DA, and their principal metabolites. Neuroradiological measures consisted of ventricular brain ratio (VBR), bifrontal/bicaudate ratio, and estimates of cortical atrophy. We also studied the effects of disulfiram, an inhibitor of DBH in selected patients with TD.

Methods Employed: Over the past six years, 111 inpatients at Saint Elizabeths Hospital, 104 meeting Research Diagnostic Criteria for schizophrenia, were studied. An effort was made to include a diverse group of patients, varying in age (19-95 years), duration of illness (1-63 years), and diagnostic subtype (primarily, chronic paranoid and undifferentiated schizophrenia). Patients were examined by two psychiatrists for the presence of TD. Forty-one patients met specific diagnostic criteria for TD, based on phenomenology, duration, relationship to neuroleptic treatment, and exclusion of other movement disorders. The severity of abnormal movements was rated in all patients with the Abnormal Involuntary Movement Scale (AIMS) (interrater reliability r_p=0.83); a global scale score of two or greater (mild, but definitely abnormal) in any of three body regions - face (AIMS-F), limbs (AIMS-L), or trunk (AIMS-T) - was also required for the diagnosis of TD.

Subgroups of patients were further evaluated with the Brief Psychiatric Rating Scale (BPRS), and assayed for plasma DBH activity (with a photo-metric assay) (interassay reliability ICC=0.81, $p<0.001$), specific platelet alpha-2 binding (with the antagonist ^{3}H-dihydroergocryptine (^{3}H-DHE) displaced by phentolamine), and lumbar CSF NE, MHPG (the "CNS contribution" to CSF MHPG was derived from the equation (CSF MHPG - 0.9 (simultaneous plasma MHPG))), DA-sulfate, HVA, and DOPAC (by HPLC with electrochemical detection). In addition, 89 patients underwent CT scans, which were assessed for

ventricular/brain ratio (VBR), bifrontal/bicaudate ratio, and cortical atrophy, using previously described methods.

All patients were studied in the steady state, primarily when neuroleptic-free for more than two weeks, or when on steady-dose neuroleptic treatment for more than four weeks. All biochemical assays and neuroradiologic assessments were performed "blind" with respect to TD status and other clinical data. In six TD patients, we studied the effects of disulfiram on plasma DBH activity and severity of TD.

Major Past Findings: Patients with TD had significantly greater plasma DBH activity than those without TD (44.4 $\pm$ 22.7 vs 30.3 $\pm$ 19.6 nmole/ml/min, T=-3.27, $p < 0.002$, t-test). This difference was not accounted for by differences in age or duration: neither variable correlated with DBH activity. Patients with TD also had a trend toward larger VBR's than those without TD (9.6 $\pm$ 5.3 vs 7.5 $\pm$ 5.4, T=-1.77, $p < 0.10$, t-test); VBR, however, was highly correlated with age (r_p=0.68, $p < 0.0001$) and any difference in VBR was lost when age was considered as a covariate (F=0.03, $p > 0.85$, Analysis of Covariance).

We did not observe a significant correlation between DBH and VBR (in all patients studied together (r_p=0.09) or in TD and non-TD patients considered individually (r_p=-0.10 and r_p=0.14, respectively)).

No other measure discriminated TD from non-TD patients. CSF-NE was increased in TD patients (1.27 $\pm$ 0.80 vs 0.84 $\pm$ 0.42 pmole/ml) but this missed statistical significance at 5% level.

Despite differences in NA indices, it is noteworthy that TD patients did not differ in any DA index measured, including CSF DA-sulfate, HVA, or DOPAC. Patients on and off neuroleptics had almost identical values for DBH (as well as NE and MHPG) and DBH did not correlate with neuroleptic dose (in mg chlorpromazine-equivalents).

The severity of orofacial dyskinesias (AIMS-F score) in patients with TD correlated significantly with CSF NE (r_s=0.93, $p < 0.01$) and with platelet ^{3}H-DHE binding (r_s=0.70, $p < 0.05$); these NA indices also appeared to be correlated with one another (r_s=0.80, $p < 0.20$). A measure of overall severity (AIMS-total = AIMS-F+AIMS-L+AIMS-T) showed no such correlation with NA indices. Of interest, neither plasma DBH nor CSF DA and its metabolites were related to symptom severity.

Plasma DBH and CSF NE were not inter-correlated. There was, however, the suggestion of a non-significant trend for plasma DBH to be correlated with the ratio NE/DA-sulfate (r_s=0.41, $p < 0.15$); this is interesting since DBH catalyzes the conversion of DA into NE.

New Findings: As a group, patients with TD had greater plasma DBH activity than those without. Yet, not all patients with TD had elevated DBH activity. We divided all subjects (both TD and non-TD) into those with DBH activities above and below the mean (35.8 nmole/ml/min). TD patients with "high" and "low" DBH activities, so defined, did not differ in age, duration, gender, race, or mean neuroleptic dose (in mg chlorpromazine-equivalents). They did not differ in abnormal movement localization or severity. They did differ in clinical psychopathology rating scores: there were higher BPRS total scores (reflecting higher positive symptom scores) in TD patients with "high" DBH activity (2.13 $\pm$ 0.47 vs 1.09 $\pm$ 0.85, $p < 0.05$, t-test). While TD patients with "high" and "low" DBH activity could in general not be differentiated, TD patients with "low" DBH activity could be separated from

non-TD patients with "low" DBH activity. The former were more likely to demonstrate enlarged VBR's on CT scan (11.23 ± 6.96 vs 7.15 ± 5.44, T=-1.98, p=0.056, t-test).

Structural brain abnormalities in "low" DBH TD patients appeared to be limited to VBR enlargement. None of the eight patients with TD demonstrated cortical atrophy, while 10 of the 25 patients without TD demonstrated such atrophy (p 0.08 two-tailed Fisher exact probability). If anything, this is in a direction opposite to the VBR findings. While this suggested that subcortical, rather than cortical, atrophy was responsible for the larger VBR in the low DBH TD group, there was no difference between low DBH TD and non-TD patients in bifrontal/bicaudate ratio. The disulfiram study showed that changes in plasma DBH activity preceded those in severity in TD, suggesting that TD per se was not the cause of elevated DBH in a group of TD patients.

In sum, a portion of patients with TD manifested either NA abnormalities (elevations in plasma DBH activity and in CSF NE) or neuroradiologic abnormalities (enlarged VBR) when compared to patients without TD. Among patients with TD, biochemical and structural abnormalities were inversely related: VBR enlargement was most apparent in TD patients with "low" DBH activity.

Thus, TD is probably not a unitary disorder, but a syndrome with heterogeneous symptomatology, and most likely, heterogeneous pathophysiology. While drug (primarily neuroleptic) treatment is a necessary factor in the development of TD, it is not the only factor. Noradrenergic (elevated DBH) and neuroanatomic (enlarged VBR) abnormalities may also be risk factors, and suggest a rational approach to subtyping and pharmacologic treatment. Absence of significant differences in CSF-dopamine and its metabolites, between TD and non-TD groups argues against the popular hypothesis of postsynaptic D2 receptor supersensitivity in TD.

Significance to Mental Health Research: Neuroleptic-induced tardive dyskinesia is a major public health concern in psychopharmacology. With a reported prevalence of over 25% among chronic inpatients, it is an important limiting factor in the use of neuroleptics in psychiatry. Improved understanding of the pathophysiology of this syndrome may help in development of newer and better treatments as well as prevention.

Proposed Course of Project: This work is being carried on for several years. We expect it to continue with emphasis on identification of specific noradrenergic and neuroradiologic abnormalities in subgroups of patients with TD. We also hope to develop pharmacologic treatments based on our findings.

Publications:

Jeste, D.V., Doongaji, D.R. and Linnoila, M.: Elevated cerebrospinal fluid norepinephrine in tardive dyskinesia. Brit. J. Psychiatry, 144:177-180, 1984.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02258-01 NPB

PERIOD COVERED

October 1, 1984 through September 30, 1985

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

Quantitative Neuropathology of Aging and Neuropsychiatric Disorders

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

Dilip V. Jeste, M.D., Staff Psychiatrist, NPB, IRP, NIMH

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Neuropsychiatry Branch

SECTION

Section on Aging

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.50	.50	

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 been studying quantitative neuronal density, neuronal size and nuclear size in selected areas of brains from patients with certain neuropsychiatric disorders as well as normal controls from different age groups. Results to date indicate that there is normally an age-related purkinje cell loss in the anterior vermis, posterior vermis and hemispheres of the cerebellum. In contrast, multipolar cells in the cerebellar dentate nucleus and hipocampal pyramidal cells (with a possible exception of those in the sector CA_4) show no significant change with aging. The reasons for such differential vulnerability of neurons to aging are still unclear, but probably include differences in catecholamine content. We found no significant differences in any of the measures related to cerebellar Purkinje cells, between controls and patients with major psychiatric disorders including schizophrenia, unipolar and bipolar disorders. There was, however, a suggestion for a decrease in Purkinje cell density related to long-term treatment with neuroleptics.

PHS 6040 (Rev. 1/84)

GPO 904-917

Project Description:

Objectives: A neuron forms the basic structural and functional unit of the nervous system. Neuronal changes are usually at the core of most gray-matter diseases of the brain. Indirect evidence implicates neuronal damage in specific areas of the brain in normal aging as well as in a number of neuropsychaitric disorders. Earlier studies of the neuropathology of schizophrenia and major affective disorders have often yielded conflicting results. One reason for this lack of uniform findings is the use of qualitative methods for studying neuropathology. We rely on the newly developed computerized neuronal imaging systems that provide objective and quantitative data. The main goals of our studies are to obtain such data in selected regions of the brain-e.g., the limbic system and cerebellum in schizophrenia and affective disorders, hippocampus in dementias, basal ganglia in movement disorders. The studies are designed to answer specific questions: e.g., Does long-term neuroleptic treatment have a cytotoxic effect on certain neurons? Do unipolar and bipolar affective disorder patients differ significantly in their neuropathologic lesions? Such information will have not only theoretical, but also potentially therapeutic implications. For our studies, we have access to one of the finest neuropathology collections in the world-viz., the Yakovlev collection at the Armed Forces Institute of Pathology.

Methods Employed: In the cerebellum we measured Purkinje cell density in anterior vermis, posterior vermis and cerebellar hemispheres, and multipolar cell density in the dentate nucleus. We also measured neuronal and nuclear cross-sectional areas. In the hippocampus we measured the pyramidal cell density in regions CA_1 through CA_4

Selection of Sections: Specific sections of individual cerebellar and hippocampal areas were selected according to specified atlas criteria.

For many subjects, a large number (40 to 60) of sections of cerebellar hemisphere from each brain were available. In order to determine the variability of Purkinje cell density in different sections from the same cerebellar hemisphere, we conducted a pilot study in 12 subjects ranging in age from 10 to 90. We examined every second section of cerebellar hemisphere (i.e., 20 to 30 sections per person), and measured Purkinje cell density in several folia in each section (as described below). There was a highly significant correlation among the values for neuronal density in folia from different sections of the same subject (intraclass correlation coefficient 0.79; $p < 0.0001$).

Measurements: All the measurements were done "blind" with respect to the clinical and autopsy data on the subject. We used Zeiss Videoplan II, which consisted of an electronic planimeter attached to a microcomputer, a light microscope, a TV camera and a video-screen. In this method, a section under microscope was magnified and projected onto a videoscreen which was, in turn, connected to a magnetic tablet and a computer. By using a cursor on the magnetic tablet, each Purkinje cell was counted once and only once. Only those Purkinje cells that displayed a distinct nucleolus were counted. Quantitative measurements of the number of neurons, and surface area and perimeter of the cerebellar folium under view were done by the computer. The convex portion of a folium was always included in the section being viewed. Purkinje cells are more closely packed over the convex part of the folium than in the deeper concave portions. We computed Purkinje cell density as the number of cells per unit area (mm^2) and unit perimeter (mm) of the folium, rather than per unit volume. We selected only those folia that showed a monolayer arrangement of Purkinje cells, and were relatively uniform in total surface area. Folia with abnormal stretching or separation of layers, were excluded.

Using a similar technique we measured the density of multipolar cells per mm^2 in the dentate nucleus. We also measured sizes in terms of projected cross-sectional areas (in mm^2) of three "typical" looking Purkinje cells from each section of anterior vermis. Sizes of the neuronal nuclei were also measured.

Finally, with the same technique we measured the density of pyramidal cells in regions CA_1 through CA_4 of the hippocampus (in cells/mm^2).

Reliability: Three investigators independently measured Purkinje cell density in selected sections from the same subjects (n=19). The intraclass correlation coefficient for the values obtained by the three raters was 0.82 ($p < 0.00001$); measurements of Purkinje cell-size, nuclear size and density of multipolar cells in the dentate also had a significant inter-rater reliability ($p < 0.0005$).

Major Past Findings: We selected two regions of the brain with considerable functional significance-viz., cerebellum and hippocampus, which are important for coordination and memory, respectively. Aging in associated with a reduction in both these functions. Also cerebellum and hippocampus have been implicated in several psychiatric disorders too.

1. Cerebellum and aging. We studied 49 cerebella from the normative series of the Yakovlev collection. We found a highly significant negative correlation between age and Purkinje cell density, especially in the anterior vermis (Pearson's r=.62, $p < 0.0001$). No correlation with age was found in Purkinje cell area or Purkinje cell nucleus area, nor with dentate nucleus multipolar cell area. No differences were observed between men (n=30) and women (n=19).
2. Hippocampus and aging. We studied right and left hippocamp; from 20 brains in the "normative series" of the Yakovlev collection-age range, 6-94. No significant correlations with age were found in hippocampal pyramid cell density nor with pyramidal cell area and nuclear area in regions CA_1 through CA_4. There were also no differences between men (n=9) and women (n=11). Subjects over age 65 had lower mean pyramidal cell density than those under 65, but this only reached significance in area CA_4 (23.2 ± 5.5 vs 29.4 ± 5.7 cells/unit area, two-tailed $t=2.101$, $p < 0.05$).
3. Cerebellum and psychiatric disorders. From the Yakovlev collection, we examined 23 brains from schizophrenic subjects (all of whom had had leukotomies) and 23 brains from leukotomized controls (7 manic-depressive, 5 unipolar depressive, 3 psychopathy, one anorexia nervosa, one psychogenic pain disorder, and 6 with chronic pain). We compared these groups with an age- and sex-matched group of 37 brains form the "normative" series for Purkinje cell density in anterior vermis and cerebellar Purkinje cell area and nuclear area, and dentate multipolar cell density. No significant differences were found between the groups on these measures.
4. Cerebellum and disorders of basal ganglia. We found a significant (about 50%) loss of Purkinje cells in 8 out of 17 patients with Huntington's disease. Patients with Parkinson's disease exhibited a less consistent loss of Purkinje cells. These findings are consistent with our clinical studies showing signs of cerebellar dysfunction in patients with Huntington's and Parkinson's diseases.

New Findings: Our studies suggest that certain areas (such as the anterior vermis) may be much more susceptible to the effects of aging than other regions (such as the dentate nucleus), in terms of neuronal loss. Our literature-review indicates a very interesting possibility that neurotransmitter content may determine, at least partially, the relative vulnerability to aging-related neuronal loss. We will pursue this line of thinking in our future studies.

Our finding of a significant loss of the large Purkinje cells in Huntington's disease challenges the traditional concept of that disease as a disorder of small neurons. Our clinical studies confirm cerebellar dysfunction in Huntingtons's disease.

We observed a significant decrease in Purkinje cell density in neuroleptic-treated patients as compared to well-matched controls. This suggests a need for further studies of neurotoxicity of these drugs. Such a finding has clinical as well as medicolegal implications.

Significance to Mental Health Research: Neuropathology is one of the most neglected areas in mental health research. This is even more true for quantitative neuropathology. The latter is important if we are to define the "sites of lesion" in disorders such as schizophrenia and major affective disorders and to understand the process of aging better.

Such studies will improve our insight into the pathophysiology of aging and various neuropsychaitric disorders, and further, lead to development of treatments that may have actions on specific brain-regions. From another perspective, such studies may also highlight neurotoxicity of currently available treatments. Quantitative neuropathology will also help in brain studies of animal modes of aging and certain neuropsychiatric disorders.

Proposed Course of Project: This work began two years ago on a small scale. We will be expanding this work with more sophisticated instruments during the next year.

Publications:

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

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October 1, 1984 through September 30, 1985

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

Peripheral and Central Catecholamine Turnover in Depression and Schizophrenia

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

Neuropsychiatry Branch

SECTION

Section on Psychopharmacology

INSTITUTE AND LOCATION

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.45	0.8	0.65

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.)*

Analytical procedures were developed for the estimation of total body turnover of norepinephrine (NE) and dopamine (DA) in both humans and experimental animals. These procedures were employed to evaluate total body NE and DA in schizophrenic and depressed patients on and off medication. To determine the relationship between total body turnover of catecholamines and brain amine turnover and metabolism, supplementary experiments were carried out on rats exposed to the same treatments as those administered to humans. The results of our findings are as follows:

1. Total body NE turnover (sum NE) is elevated in major depression with melancholia but not in depression without melancholia.
2. Five forms of antidepression treatments (low doses of clorgyline, desipramine (DMI), zimelidine (ZMI), electroconvulsive treatment (ECT) and lithium) significantly reduced sum NE. Sum DA was not consistently reduced.
3. There is a tendency for sum DA but not sum NE to be reduced in chronic schizophrenia. When sum DA and sum NE were evaluated, chronic schizophrenic patients had significantly lower ratios than sex and age matched controls. Haloperidol treatment normalized these ratio.
4. Consistent with human analyses, DMI, ZMI and lithium chronic treatments significantly reduced sum NE in rats as well as in the hypothalamus, suggesting a positive correlation between total body turnover of NE and brain NE turnover. ECT significantly increased sum NE and brain NE turnover. These four types of antidepressants produced inconsistent changes in sum DA and caudate nucleus turnover of DA. DMI and ZMI, significantly reduced caudate nucleus DA turnover suggesting some role of brain DA in the therapeutic actions of these two drugs.
5. As part of our exploratory studies we have also evaluated the in vivo effects of 3 classes of monoamine oxidase inhibitors (MAOI) on central and peripheral catecholamines. Our results indicated a good correlation between changes in sum NE and sum DA and those occurring in the brain. The three MAOIs employed were pargyline, clorgyline and deprenyl.

Collaborators:

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Project Description:

Objectives: Central biogenic amines especially norepinephrine (NE), dopamine (DA) and serotonin (5HT) are believed to be involved in the etiology of some mental disorders especially schizophrenia and depression. Unfortunately there is a lack of direct evidence supporting this view. Thus although there exist good circumstantial evidence of disturbed brain DA in schizophrenia and of an imbalance in central NE and 5HT in depression, efforts to directly establish abnormal production of these amines or their metabolites in these disorders are best described as suggestive but not entirely convincing.

As part of our long term interest in the role of biogenic amines in mental illness and in the pharmacologic properties of drugs employed in their treatments, we embarked on a project expected to offer better insight into the disposition of catecholamines in depression and schizophrenia. The strategy taken falls under three categories.

1. To develop analytical methods that are accurate, sensitive and highly reproducible.
2. Employ these methods to measure urinary excretion of catecholamines and their metabolites in depression and schizophrenia.
3. To use rats as supplements to determine the relationship between total body amine turnover and central changes observed in humans after medication.

Methods Employed: All biochemical analyses were carried out by combined gas chromatography mass spectrometry employing the technique commonly referred to as mass fragmentography. At least two 24 hour urine collections were analyzed from each subject. All biochemical analyses were carried out on coded samples. Total body NE and DA turnovers were measured by adding up the molar excretions of the amines and their appropriate metabolites.

Findings: Modifications of the biochemical methods employed have been applied to the assay of catecholamines and other biogenic amines in biological materials from both humans and rats. These studies have confirmed the report of increased NE concentration in the nucleus accumbens of paranoid schizophrenic patients. In addition we have observed an increase in hypothalamic concentrations of NE and its major metabolite 3-methoxy-4-hydroxyphenylglycol (MHPG) in paranoid schizophrenics. Futhermore, we have assessed the effect of chronic phenylethylamine (PEA) treatment on rat brain NE and DA turnover. Our result indicated an increase in hypothalamic and nucleus accumbens NE and MHPG concentrations, since PEA is suspected to be an endogenous amphetamine-like substance that may be responsible for some of the symptoms of schizophrenia. The results of our human postmortem brain analyses and those of the effects of PEA on rat brain NE suggest some association between the increase in nucleus accumbens NE and MHPG concentrations in chronic paranoid schizophrenia and increase production of brain PEA. This is because PEA excretion tends to be elevated in some schizophrenic patients.

Five types of antidepressant treatments commonly employed in the management of affective disorders were found to reduce total body turnover of NE. These treatments include low dose clorgyline, desipramine (DMI), zimededine (ZMI), electroconvulsive treatment (ECT) and lithium carbonate (Li). DA turnover was reduced after clorgyline and Li, but remained unchanges after DMI, ZMI and ECT. Additionally, unipolar depressed patients were found to excrete lower amounts of MHPG as compared to normal subjects.

To determine how changes in urinary excretion rates of catecholamines and their major metabolites correlate with central amines, we treated rats chronically with DMI, ZMI, ECT

and Li and measured sum NE, sum DA and brain turnover of NE and DA. The results obtained showed correlations with changes observed in sum NE in the depressed patients. ECT elevated sum NE in rats which is opposite to what was found in depression. All four treatments except Li, failed to change sum DA in rats.

Lithium carbonate significantly reduced NE turnover in the hypothalamus. ECT significantly increase hypothalamic NE turnover. Thus the changes observed in sum NE after the treatments are parallel to those found in the hypothalamus.

AD for brain DA, DMI and ZMI but not ECT or Li, significantly reduced hypothalamic and caudate nucleus DA turnover rate suggesting the involvement of brain dopaminergic neuronal systems in the therapeutic benefits of DMI and ZMI.

Chronic schizophrenic patients off medication for at least 4 weeks exhibit a strong tendency for a reduced total body DA turnover. NE turnover appears to be normal; a situation opposite to what was encountered in depression. The tendency for sum DA to be low in chronic schizophrenia resulted in significantly lower sum DA and sum NE in chronic schizophrenia as compared to gender and age matched controls.

Our findings suggest 1) That depression is related to a hyperactive noradrenergic system and that antidepressant treatments induced a lowering in NE turnover rather than an elevation. This latter effect is consistent with the hypothesis of down regulation of -noradrenergic receptors by antidepressants and, 2) That schizophrenia appears to be associated with hypodopaminergic systems rather than the prevailing view that it is associated with increased brain dopamine activities.

Significance to Mental Health Research: Our findings have established in a consistent manner that treatments frequently employed for the management of depression are primarily targeted towards body noradrenergic systems. Supplementary experiments on rats indicated that changes in total NE turnover after antidepressants are similarly reflected upon brain especially hypothalamic NE turnover and metabolism. Therefore the procedures that we have employed to carry out our studies are expected to prove valuable in clinical investigations aimed at assessing catecholamine disposition in mental disorders including depression and schizophrenia. The use of the rat to supplement human studies is also expected to prove useful.

Proposed Course of Project: We plan to employ the procedures developed and described here to conduct more studies on the pharmacological effects of antipsychotic drugs on total body NE and DA turnover in both humans and rats. We will continue to use the rat to correlate total body amine turnover with central amine turnover and metabolism. We also hope to include such amines as phenylethylamine, tyramine and indole amines to our future investigations.

Publications:

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Chen, P.H., Tilson, H.A., Marbury, G.D., Karoum, F. and Hong, J.S.: Effect of chlordecone (Kepone) on the rat concentration of 3-methoxy-4-hydroxyphenyglycol: Evidence for a possible involvement of norepinephrine system chlordecone-induced tremor. Toxicol. Appl. Pharmacol., 77:158-164, 1985.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

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PERIOD COVERED

October 1, 1984 through September 30, 1985

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

Blink Rate in Neuropsychiatric Disorders

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

Craig Karson, M.D., Medical Officer, Neuropsychiatry Branch, IRP, NIMH

Dr. Joel E. Kleinman, Chief, Section on Clinical Brain Studies, NPB, IRP, NIMH; Dr. Karen F. Berman, Staff Psychiatrist, NPB, IRP, NIMH; Dr. Terry L. Goldberg, Michigan Osteopathic Medical Center, Child and Youth Mental Health Hospital; Jimmie P. Leleszi, D.O., Tourette and Tic Laboratory and Clinic, Mount Sinai Medical Center, N.Y.; Dr. Arthur K. Shapiro, Tourette and Tic Laboratory and Clinic, Mount Sinai Medical Center, N.Y.; Dr. Elaine Shapiro, Tourette and Tic Laboratory and Clinic, Mount Sinai Medical Center, N.Y.

COOPERATING UNITS *(if any)*

Michigan Osteopathic Medical Center, Child and Youth Mental Health Hospital; Tourette and Tic Laboratory and Clinic, Mount Sinai Medical Center, New York; Laboratory of Experimental Therapeutics, NINCDS

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Clinical Brain Studies Section

INSTITUTE AND LOCATION

NIMH, NIH, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.80	.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 National Institute of Mental Health in conjunction with the Laboratory of Experimental Therapeutics, NINCDS; Michigan Osteopathic Medical Center, Child and Youth Mental Health Hospital and the Tourette and Tic Laboratory and Clinic of Mount Sinai Hospital are examining of blink rate in a variety neuropsychiatric disorders. In medication-free patients with movement disorders blink rate is decreased in those patients with parkinsonism or progressive nuclear palsy but not increased in several hyperkinetic states including dystonia, tardive dyskinesia and Tourette's syndrome. In the parkinsonian patients the blink rate correlates with the severity of the illness. Patients with schizophrenia who have been withdrawn from medication have a slight but statistically significant increase in their blink rate. This increase is primarily seen in the spring-summer period. Moreover, never medicated adolescent inpatients with "psychosis" have an increased blink rate compared to their nonpsychotic counterparts, indicating that increased blinking may precede treatment. Finally compared with normal children of the same age blink rate is increased in autistic children but decreased in children with mental retardation. We have attempted to link blink rate to central dopamine activity and explain these differences with regard to alterations of dopamine activity in the various disorders.

PHS 6040 (Rev. 1/84) GPO 904-917

Collaborators:

Dr. Charles A. Kaufmann, Neuropsychiatry Branch, IRP, NIMH

Project Description: ZO1 MH 02260-01 NPB

Objectives: Spontaneous eye-blink rate is non-invasive and easily measured. The classical finding of reduced blink rate in Parkinson's disease, in which there is also destruction of midbrain dopaminergic systems, led to the hypothesis that blinking may be modulated by brain dopamine activity. Increased blink rate in monkeys injected with the dopamine agonist apomorphine strengthed the "dopamine hypothesis" of blink rate. The primary objective of this investigation was to investigate eye-blink rate in a variety of neuropsychiatric conditions to see what abnormalities of blink rate exist and if existing evidence or hypotheses about brain dopamine activity in a given disorder could help predict the direction of change in blinking. Ultimately, it was hoped that blink rate could provide a noninvasive probe of brain dopamine activity.

Methods: Blink rate is determined through direct observation by an observer equipped with a stop watch, except in Tourette's syndrome where the rate was determined by viewing videotapes of patients. In general, blinks were counted for 3 consecutive 1 minute periods at a frequency of not more than 1 count per week.

In conjunction with the Laboratory of Experimental Therapeutics, NINCDS, blink rate was observed in 25 medication-naive patients with Parkinson's disease (mean age 54 $\pm$ 4 years), five patients with progressive supranuclear palsy (PSP) (mean age 55 $\pm$ 11 years), 10 patients with Huntington's chorea withdrawn from all medications (mean age 47 $\pm$ 14 years) and nine medication-free subjects with dystonia (mean age 41 $\pm$ 8 years). In addition, in a separate collaboration with the Tourette and Tic Laboratory of Mount Sinai Hospital videotapes of another group of hyperkinetic patients, those with Tourette's syndrome were made available. Recordings of 19 Tourette's patients (mean age 24 $\pm$ 12 years) on and off pimozide during different cognitive tasks were examined and the rates for the patients compared to that seen in a large group of age matched normals counted by direct observation.

Two broad categories of psychiatric patients, children and adults, have also been studied. Two studies have been carried out at Michigan Osteopathic Medical Center, Child and Youth Mental Health Hospital. In the first of these blink rate in 13 medication-free inpatients (mean age 16 $\pm$ 1) fulfilling DSM III criteria for schizophrenia, schizophreniform psychosis or atypical psychosis was compared to that of 35 other inpatients (mean age 14 $\pm$ 2 years) who were not psychotic. In a second study blinking was examined in 15 children with autism (mean age 9 $\pm$ 3 years), 34 children with mental retardation (mean age 8 $\pm$ 3 years) and 52 normal children (mean age 9 $\pm$ 2 years). The diagnosis of autism met the diagnostic criteria of the National Society for Autistic Children and 13 had DSM III discharge diagnosis of the same.

The final investigations have been carried out at the Neuropsychiatry Branch of the NIMH. At this point we have observed blink rates from 77 chronic schizophrenic inpatients withdrawn from medications (mean age 30 $\pm$ 6 years) and 96 normals (mean age 39 $\pm$ 18) from our staff or normal volunteers from the Laboratory of Experimental Therapeutics, NINCDS and who at various stages served as normal controls for each subject group. In addition, blinking was examined in 15 non-schizophrenic patients with various psychoses who were also withdrawn from all medications.

Major Past Findings: The first group of findings comes from work with animals. In monkeys in actions of apomorphine caused a dose related increase in blink rate as did atropine. Drugs affecting nor adrenergic systems such as clonidine and phenylephrine had no effect nor did the serotonin receptor antagonist LSD.

Rats have an intrinsically slow blink rate (2 ± 0.5 blinks/minute). While searching for a rodent with a more rapid rate (there are none) we found that rats who had undergone cerbellectomy had a blink rate of 8 ± 1 blink/minute. Moreover, as in monkeys, apomorphine stimulated blinking in rats.

A second group of early studies was done with normal controls to examine the effect of cognitive tasks on blink rate. Compared with the state of quiet sitting, speech and memorization increased blinking while visual fixation decreased blinking. In general, subjects with neuropsychiatric disorders are counted in the speaking state and their blink rate compared with speaking normals.

The third set of findings related to patients with Parkinson's disease who were receiving dopamine agonist therapy. Two findings emerged from studying blinking in this group; 1) Parkinsonian patients with more severe symptoms had lower blink rates; and 2) Levodopa induced dyskinesia was associated with a relative increase (100%) in blink rate in these patients.

The final set of past findings involves patients with schizophrenia. We found an elevated blink rates in patients with schizophrenia withdrawn from medications which was normalized by neuroleptic treatment.

New Findings: With an N of 77 medication-free patients with chronic schizophrenia it has become clear that the blink rate increase in the speaking condition in this disorder is only slightly, though significantly higher than normal controls (15%). One other point is that neuroleptics do not reduce blink rates in patients with large cerebral ventricles. In examining other chronic psychotic patients blink rate has been found elevated to a more significant degree. Hence elevated blink rate may be a feature of psychosis in general. To examine whether increased blinking in these chronic psychotic patients related to treatment effects blinks were examined in adolescent psychotic patients who were medication-naive. Blink rate was elevated in this group indicating that elevated blink rate in psychiatric patients precedes treatment.

In autism which has been postulated to be a form fruste of schizophrenia and which may also involve elevated brain dopamine activity, blink rates are increased. In contrast, other patients with mental retardation have decreased blink rate.

In movement disorders we have confirmed that blink rate is decreased in Parkinson's disease and PSP. Most hyperkinesias do not demonstrate elevated blink rate. It is clinically relevant that patients with more severe Parkinson's disease have a lower blink rate.

Significance to Mental Health Research: Insofar as blink rate reflects brain dopamine activity, increased blinking in schizophrenia, other psychosis and autism may point to a role for increased dopamine activity in these conditions. The decrease blink in Parkinson's disease and the relative increase in levodopa induced dyskinesia further suggest a role for blink rates as a monitor of neuroleptic-induced extrapyramidal side effects and perhaps for the therapeutic efficacy of neuroleptics in a particular patient.

Increased blink rate in normal controls who are memorizing and decreased blink rate in patients with mental retardation may indicate that blink rate may be of use in understanding cognitive disorders.

At any rate, it is hoped that blink rate with cognitive and neurochemical correlates can serve as a useful non-invasive probe of brain dopamine activity.

Proposed Course of Project: In the future studies of blink will concentrate on patients with cognitive disturbances such as Korsakoff's disease and Alzheimer's disease. In addition we will begin to examine blink in other psychiatric conditions to see if elevated blink rate occurs only in psychosis. In addition, hopefully animal experiments may be undertaken to further elucidate the neuroanatomy of blink rate.

Publications:

Karson, C.N., Burns, S., LeWitt, P.A., Foster, N.L. and Newman, R.P.: Blink rates and disorders of movements. Neurology, 34:677-678, 1984.

Karson, C.N., Berman, K.F., Kleinman, J.E. and Karoum, F.: Seasonal variation in human central dopamine activity. Psychiatry Res., 11:111-117, 1984.

Kleinman, J.E., Karson, C.N., Weinberger, D.R., Freed, W.J., Berman, K.F. and Wyatt, R.J.: Eye-blinking and cerebral ventricular size in chronic schizophrenic patients. Am. J. Psychiatry 141:1430-1432, 1984.

Karson, C.N., Kaufmann, C.A., Shapiro, A.K. and Shapiro, E.: Eye-blink rate in Tourette's syndrome. J. Nerv. Ment. Dis., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 02261-01 NPB

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Clinical Phenomena in Schizophrenia: Quantification in a Effort to Subtype

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

Craig N. Karson, M.D., Staff Psychiatrist, NPB, IRP, NIMH

Dr. Llewellyn B. Bigelow, Associate Clinical Director for Research at Saint Elizabeths, IRP, NIMH; Dr. Dilip V. Jeste, Medical Officer, NPB, IRP, NIMH; Dr. William B. Lawson, Staff Psychiatrist, NPB, IRP, NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH
Neuropsychiatry Branch

SECTION
Section on Clinical Brain Studies

INSTITUTE AND LOCATION
NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.20	.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.)*

To delineate potential discreet subgroups of patients with schizophrenia we have studied quantifiable psychopathological, behavioral, and physiological variables in schizophrenic patients. In terms of psychopathology we have done two studies: (1) Constructed a quotient of two item scores from the BPRS as follows: suspiciousness/(disorganized speech + suspiciousness) x 100. Patients with paranoid schizophrenia had a higher quotient score (70) than other patients with schizophrenia (47, $p < .002$). (2) To examine whether psychopathology may be related to the expression of TD in schizophrenia we examined BPRS syndrome clusters in patients with schizophrenia. Patients with TD had a preponderance of high depression and negative symptoms scores but lower anxiety scores. Behaviorally we are concentrating on the issue of aggression in schizophrenia and have found that patients with schizophrenia are more aggressive than are other research patients (> 40% committed physical assaults on others) and that assaultiveness tended to be associated with reduced cerebrospinal fluid (CSF), norepinephrine (NE), and MHPG. A final physiological variable we have studied is urine volume. The mean volume of urine of patients with schizophrenia is twice that of normal controls (2300 vs 1300 ml/24 hr).

PHS 6040 (Rev. 1/84) GPO 904-917

Project Description:

Objectives: Since 1974 psychopathological, behavioral, and physiological data has been gathered from a large number of research volunteers. During this same time the interest in the neurobiology of schizophrenia has intensified and our collection of neurobiological material from these same subjects has continued. The recognition that schizophrenia may be a heterogeneous disorder has led to a constant vigil on our part so that variables from the psychopathological, behavioral and physiological realms might help to subgroup patients and potentiate finding the neurological substrates of this disorder.

Findings:

(1) Psychopathology

(a) The BPRS averaged on a daily basis from the final two weeks of a period of medication-withdrawal from 56 RDC (+) patients with chronic schizophrenia was the data base. A quotient constructed from two items, suspiciousness/(disorganized speech + suspiciousness) x 100 was significantly higher in patients with paranoid schizophrenia (70 vs 47, $p < .002$) whereas the items alone were less discriminatory.

(b) Again the BPRS from the final two weeks of a period of medication withdrawal was used as the data base, this time from 47 patients with RDC (+) chronic schizophrenia. Seventeen of these patients had tardive dyskinesia (TD). When considering proportionality about the median, patients with TD had more patients above the median score for depression syndrome, withdrawal syndrome and more below the median for the anxeity syndrome then patients without dyskinesia. This was particularly true in patients who had more persistent TD.

(2) Behavioral

Between January 1974 and March 1985, 140 patients were admitted to and discharged from the William A. White Building, staying at least one month. Ninty-seven of these patients met diagnostic criteria for schizophrenia. Fourty-one/97 patients with schizophrenia became physically assaultive while only 4/43 non-schizophrenic patients did. A majority of the assaults occurred during research related perturbation of medication status. The mean age of the violent patients was four years, younger (27 vs 31, $p < .05$) and those with a history of violence were more likely to be violent though this relationship was far from absolute. In 33 patients in whom CSF was obtained there was a tendency for reduced NE concentrations ($p < .1$) and a significant reduction in its metabolite MHPG ($p < .05$).

(3) Physiological

Over a six-year period urine was collected from 28 patients during a period of medication withdrawal. The mean volume for patients (2319 ml/24 hr) exceeded that of controls (1265 ml/24 hr, $p < .03$). Neuroleptic treatment increased urine volume ($p < .05$). In the three polyuric patients with serum sodium < 136 mEq/L. TD and hypertension was found.

Significance to Mental Health Research: (a) The paranoid quotient provides a method to quantify the concept of paranoid schizophrenia and might provide an objective numerated parameter to test hypotheses concerning paranoia.

(b) High depression and withdrawal syndrome scores in patients with TD may help to unravel who is vulnerable to this side effect of neuroleptics.

(c) Violence is a social problem of enormous consequence. Patients with schizophrenia do become violent freqeuntly. A careful study of violence in this disorder may help sort out the neurobiological substrates common to both problems.

(d) Many patients with schizophrenia die unexpectedly. Undoubtedly water intoxication is one mode of unexpected death. Hence, epidemiological studies are crucial to sort out which patients might suffer from this problem. Moreover, patients with water intoxication may be more vulnerable to TD.

The overall significance to these projects, however, is to reemphasize the importance of careful clinical observation and studies in patients with schizophrenia.

Course of Project: All of these projects are ongoing. Since violence can be studied in human brains both in terms of neuropathology and neurochemistry we are attending to the history of violence in patients with schizophrenia in whose brains we have obtained to examine potential neurobiological correlates from post mortem studies. We are also obtaining the brains of violent offenders for comparisons.

Publications:

Lowson, L.B., Karson, C.N., Bigelow, L.B. and Wyatt, R.J.: Polyuria and polydipsia in schizophrenia. Psychiatry Res. 14:323-331, 1985.

Karson, C.N., Jeste, D.V. and Bigelow, L.B.: Tardive dyskinesia and psychopathology in chronic schizophrenia: A cross-sectional study. Compr. Psychiatry, in press.

Karson, C.N. and Bigelow, L.B.: The paranoid quotient: A BPRS ratio for exploring subtypes in schizophrenia. Acta Psychiatr. Scand., in press.

Karson, C.N., Kleinman, J.E. and Wyatt, R.J.: Biochemical conceptions of schizophrenia. In Millon, T. and Klerman, G. (Eds.): Comtemporary Issues in Psychopathology. New York, The Guilford Press, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02262-01 NPB

PERIOD COVERED

October 1, 1984 through September 30, 1985

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

Electroretinography in Schizophrenia

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

Craig N. Karson, M.D., Neuropsychiatry Branch, IRP, NIMH

Dr. Karen Faith Berman, Staff Psychiatrist, NPB, IRP, NIMH; Dr. Alec C. Roy, Medical Officer, Neuroscience Branch, NIMH; Myles J. Jaffe, O.D., National Eye Institute, NIMH; Dr. Francisco M. DeMonasterio, Chief, Section on Visual Processing, National Eye Institute, NIH

COOPERATING UNITS *(if any)*

Section on Visual Processing
National Eye Institute, NIH

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Clinical Brain Studies Section

INSTITUTE AND LOCATION

NIMH, NIH, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.75	.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 National Institute of Mental Health together with the Section on Visual Processing of the National Eye Institute are involved in an ongoing study of possible electroretinographic abnormalities in patients with schizophrenia. Two separate studies have been completed. The only replicable finding has been an increased latency in the blue cone response. In addition, since we have expanded our study of rods in the second study, it appears that at low intensity stimulation patients with schizophrenia may have decreased amplitude rod responses.

PHS 6040 (Rev. 1/84) GPO 904-917

Project Description:

Objectives: The electroretinogram (ERG) is an evoked potential from the retina generated when light strikes the retina. It has three main components, an A wave, a B wave, and several oscillations about the B wave termed oscillatory potentials (OP's). Several studies in various classes of animals have shown that pharmacological manipulations of dopaminergic systems can alter the amplitudes or latencies of the B wave and OP's, though the direction of alteration has not been consistent throughout species.

Since the retina is embryonically derived from brain and regarded as CNS, and the ERG may be effected by perturbations of dopamine, we undertook an investigation of the ERG in schizophrenia, in which abnormalities of brain dopamine activity have been proposed.

Methods Employed: After a period of dark adaptation of one half hour, a subject is seated with his chin resting on a hollowed out globe called a ganzfield. This globe has been specially prepared so that a light flash from the photostimulator situated at the top of the globe is reflected evenly throughout the globe. All areas of the retina are stimulated equally. By adjusting the background light color and using a colored wratten filter to color the light flash, it is possible to stimulate rods, red-green cones, blue cones and all cones separately.

We conducted two consecutive studies. The first consisted of 15 patients with chronic schizophrenia (mean age 30 $\pm$ 6 years) and two normal controls (mean 28 $\pm$ 5 years). In an attempt to replicate results from this first study we conducted a second study of 13 patients with schizophrenia (mean age 29 $\pm$ 7 years) and 20 normal controls (mean age 24 $\pm$ 3 years). All patients were studied during a period of medication withdrawal of at least two weeks and when they returned to neuroleptic treatment. Patient values for a particular parameter, were compared with controls using a Mann-Whitney U test with a corrected p value for the large number of comparisons made.

Major Past Findings: In our first study we found that patients with schizophrenia had both increased latency and amplitude in the blue cone b wave.

New Findings: In the second study we were only able to replicate the increased latency of blue cone b wave. We were unable to detect any effect of neuroleptics on the ERG or clinical factors such as diagnostic subtype or the presence or absence of tardive dyskinesia.

Significance to Mental Health Research: One of the greatest difficulties in treating and studying psychiatric illness is our inability to see into the brain. The importance of the electroretinogram (ERG) is that it is a direct but noninvasive means of seeing into the functions and activity of the central nervous system.

Proposed Course of Project: The "flash" ERG that we have thus far utilized involves photoreceptors and related systems, and has proved tantalizing if unrewarding. A different type of ERG called pattern ERG, stimulates retinal ganglion cells, one level above the photoreceptors. Since this also provides a direct non-invasive glimpse of one CNS function we are going to measure these pattern ERG's in patients with schizophrenia to detect any potential abnormality.

Publications:

Karson, C.N., Berman, K.F., Roy, A.C., Jaffe, M.J. and De Monasterio, F.M.: Schizophrenia: Altered biological responses to light. Ann. N.Y. Acad. Sci., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 02263-01 NPB

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Haloperidol Pharmacodynamics and Clinical Response in Schizophrenia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Darrell G. Kirch, M.D., Senior Staff Fellow, NPB, IRP, NIMH

Dr. Esa Korpi, Alcohol Research Lab., Helsinki, Finland; Dr. Llewellyn Bigelow, Clinical Director, William A. White Division, IRP, NIMH; Dr. Joel E. Kleinman, Chief, Section on Clinical Brain Studies, NPB, IRP, NIMH; Dr. Grant Ko, Senior Staff Fellow, NPB, IRP, NIMH; Dennis T. Costakos, NPB, IRP, NIMH; Dr. Richard Jed Wyatt, Chief, Neuropsychiatry Branch, IRP, NIMH; Dr. Markkuu Linnoila, NIAAA, NIMH; Dr. Bruce Phelps, NPB, IRP, NIMH; Dr. Richard Wagner, Staff Psychiatrist, NPB, IRP, NIMH

COOPERATING UNITS *(if any)*
Alcohol Research Lab., Helsinki, Finland; NIAAA, NIMH; University of Colorado Health Sciences Center, Denver, Colorado

LAB/BRANCH
Neuropsychiatry Branch

SECTION
Psychopharmacology Section

INSTITUTE AND LOCATION
NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.15	1.15	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 National Institute of Mental Health (NIMH) is studying concentrations of the drug haloperidol in the blood and post mortem tissue samples of patients with schizophrenia. A sensitive, reliable, and specific assay has been developed to measure concentrations of both haloperidol and its reduced metabolite. One study using this assay has shown that it may avoid some types of interference which complicate radioreceptor methods of determining haloperidol. A basic science study was completed that showed that the reduced metabolite of haloperidol appears not to be pharmacologically inactive. Research using postmortem brain tissue specimens has indicated that both haloperidol and reduced haloperidol may remain present in detectable amounts as long as 72 days after withdrawal from haloperidol. Clinical studies in patients with chronic schizophrenia have indicated that above a certain threshold concentration of haloperidol (approximately 5 ng/ml) in serum, a plateau exists and clinical response is not enhanced by attaining higher concentrations. This implies that in the treatment of chronic schizophrenia there is usually no additional clinical benefit to the use of very high doses of haloperidol.

PHS 6040 (Rev. 1/84) GPO 904-917

Collaborators:

Dr. Steven Zalcman, Staff Psychiatrist, NPB, IRP, NIMH
Dr. M.R. Palmer, University of Colorado Health Sciences Center, Denver, Colorado
Dr. M. Egan, University of Colorado Health Sciences Center, Denver, Colorado

Project Description:

Objectives: Although haloperidol has been in widespread clinical use for nearly 30 years, the technical ability to quantify its concentration in blood and tissue samples is a relatively recent development. To date no clear consensus has been reached regarding the clinical interpretation of blood neuroleptic concentrations. Haloperidol, one of the most frequently prescribed drugs in the inpatient treatment of schizophrenia, has been perhaps most intensively studied in this regard. There have been some reports of an inverted "U-shaped" curve for clinical improvement versus haloperidol concentration, indicating that low and high concentrations are associated with poor clinical response in comparison with an apparently optimal middle range of concentrations. Other investigators have focused on the issue of neuroleptic metabolites. Haloperidol is coverted to a reduced metabolite, high concentrations of which are reported by some investigators to be associated with poor clinical response.

The overall goal of this project has been to develop a reliable and sensitive method to quantify haloperidol and its reduced metabolite and to apply this method to basic and clinical studies of haloperidol pharmacodynamics and the treatment of schizophrenia. We have initiated, and in part completed, a series of studies involving quantitative assays, animal models, post-mortem human brain tissue, and the clinical treatment of schizophrenic subjects in an attempt to address several questions. What is the best method to quantify haloperidol and its reduced metabolite? Is reduced haloperidol pharmacologically active? Can haloperidol be quantified in post-mortem human brain tissue and how long after the discontinuation of treatment does it remain measureable? What is the association among clinical response, serum haloperidol and reduced haloperidol concentrations?

Methods Employed: A high performance liquid chromatography (HPLC) assay was developed in which a liquid/liquid extraction process allowed reliable isolation of haloperidol and reduced haloperidol from serum, plasma, and red blood cells (RBC). This assay uses chlorohaloperidol as the internal standard. Split duplicate samples run on the same day and different days yeilded coefficients of variation for both compounds below 10% at a concentration of 10 ng/ml.

Results by HPLC were then correlated with other quantitative methods, including gas chromatography (GC), radioimmunoassay (RIA), and a radioreceptor assay (RRA).

In vivo studies were performed in rats using single cell recording and micropressure ejection of both haloperidol and reduced haloperidol from multi-barrelled pipettes. This allowed study of the agonist and antagonist effects of both drugs on both caudate and cerebellar neurons.

We collected of serial "fall-off" samples over the 48 hours after the last 0.2 ng/kg dose. Other clinical data available from these patients were ratings of tardive dyskinesia, course of illness, tobacco use, and anticholinergic treatment.

Major Past Findings: The assay is sensitive to as little as 2 ng/ml using a 2 ml sample. Coefficients of variation were 4% and 7% for haloperidol and reduced haloperidol respectively, each at a concentration of 10 ng/ml. HPLC results for haloperidol correlated well with results obtained by GC (Pearson $r=0.76$, $p<0.02$) and RIA (Pearson $r=0.76$, $p<0.02$).

A subsequent study compared haloperidol determinations by HPLC and by RRA. The latter tended to give higher apparent concentrations than obtained by HPLC. Further data analysis on these samples allowed quantification of haloperidol in patients who were

medicated at the time of death or who had been withdrawn from haloperidol in the weeks preceeding death.

Clinical studies have been conducted using volunteer research subjects meeting DSM III criteria for chronic schizophrenia who were inpatients on the wards of the Neuropsychiatry Branch at Saint Elizabeths Hospital. These potients were withdrawn from medication in double-blind fashion and maintained drug-free. If their psychosis worsened, they were then changed in blind fashion to a fixed dose of haloperidol 0.4 mg/kg/day. After their first 0.2 mg/kg dose, serial blood samples were collected over 48 hours prior to starting twice a day administration of the fixed dose. Steady state samples were collected at 2,4, and 6 weeks of fixed dose treatment. Trained nursing staff performed daily Brief Psychiatric Rating Scale (BPRS) assessemnts. Some patients that had been stabilized on a fixed dose of haloperidol underwent a second drug-free period. This indicated that reduced haloperidol may interfere with RRA determinations of haloperidol, resulting in higher apparent values.

In an attempt to further define the pharmacological activity (if any) of reduced haloperidol, an in vivo study using single cell recording of rat neurons was completed. Using micropressure ejection of drugs from a four-barrelled pipette it was determined that reduced haloperidol has no agonist or antagonist properties in recordings from caudate and cerebellar neurons.

Data from haloperidol determinations on post-mortem brain tissue samples have also been published. It was found that reduced haloperidol was present in cortical tissue from treated patients at slightly higher concentrations than haloperidol itself. Haloperidol was detectable in a patient who had been withdrawn from medication for two weeks prior to death, and trace amounts were present in a patient who had been drug free for 72 days.

A major thrust in this project has been to better define the relationship between clinical response and serum haloperidol concentrations. Results of a study in which acutely decompensated schizophrenic subjects were treated in double-blind fashion with a fixed dose of 0.4 mg/kg/day have been analyzed. There was no significant correlation between clinical response and serum concentrations ranging from 6.8 to 39.4 ng/ml. We failed to find a "therapeutic window" as reported by some other laboratories, although these other reports included relatively few subjects with concentrations above 15 ng/ml in contrast to our own study which had 15 such subjects. Our data also showed no enhancement or impairment of response in those patients with higher reduced haloperidol concentration, a finding consistent with our previous basic science study of reduced haloperidol.

New Findings: Pharmacokinetic analysis of response to a single oral dose of 0.2 mg/kg of haloperidol is being performed. Preliminary data in 19 subjects indicates a significant correlation ($r=0.62$, $p < 0.01$) between the serum concentration of haloperidol 5 hours after this acute dose and the ultimate steady state concentration on a dose of 0.4 mg/kg/day.

Samples have also been collected when patients who were on the fixed dose went "drug free". These will be used to measure steady state concentration and "fall off" pharmacokinetics. Correlations between these drug concentrations and subsequent clinical relapse will be assessed.

Plasma and red blood cell samples have been collected from patients treated with a fixed dose of haloperidol. There have been reports from other laboratories of a correlation between red cell haloperidol concentration and clinical response. These samples will be used in an attempt to replicate these earlier studies.

Although our previous studies yielded no evidence that reduced haloperidol is pharmacologically active, this metabolite does remain of interest because of data analysis in progress. It appears that as haloperidol dose is increased, the ratio of reduced haloperidol to haloperidol in the serum increases. This may in part explain the clinical observation that, in many cases, higher doses of haloperidol do not improve clinical response in the chronic patient.

Significance to Mental Health Research: Although neuroleptic drugs such as haloperidol are a mainstay in the treatment of schizophrenia, dose adjustment has all too often been based on habit or custom rather than scientific rationale. The results of our studies, together with reports from other research groups, indicate that higher concentrations of haloperidol yield no additional clinical benefit in the long-term treatment of schizophrenia. It appears that a steady state serum haloperidol concentration of 10 ng/ml yields optimal response. Moreover, the pharmacokinetic studies in progress may allow prediction of steady state concentration from a single blood sample drawn after an acute dose. The goal of this project is to ultimately provide a method of monitoring neuroleptic dosing that will maximize patient response and minimize the risk of drug exposure.

Proposed Course of Project: We plan to utilize haloperidol concentration monitoring as a guide for adjusting dose in chronic schizophrenic patients on the general wards of Saint Elizabeths Hospital. This will allow confirmation, in a more clinically oriented setting, of our previous finding that a serum concentration of approximately 10 ng/ml is optimal for these patients.

We also plan to pursue the possibility that serum haloperidol concentration may be associated with side effects, specifically Parkinsonism and tardive dyskinesia.

Insofar as smoking has been identified as lowering blood concentrations of some neuroleptics, we will also compare smokers and non-smokers on a fixed dose of haloperidol.

In summary, we will continue to pursue a wide range of clinical pharmacological problems in the treatment of schizophrenia with haloperidol.

Publications:

Kirch, D.G., Palmer, M.R., Egan, M. and Freedman, R.: Comparison of haloperidol and its reduced metabolite: electrophysiological interactions with dopamine, norepinephrine, and phencyclidine. Neuropharmacology, in press.

Kirch, D.G., Bigelow, L.B., Korpi, E.R., Wagner, R.L., Zalcman, S. and Wyatt, R.J.: Serum haloperidol concentration and clinical response in schizophrenia. J. Clin. Psychiatry, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02264-01 NPB

PERIOD COVERED

October 1, 1984 through September 30, 1985

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

Post Mortem Brain Tissue Examination in Psychiatric Disorders

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

Joel E. Kleinman, M.D., Ph.D., Chief, Clinical Brain Studies, NPB, IRP, NIMH

Dr. H. Stefan Bracha, Medical Staff Fellow, NPB, IRP, NIMH; Dr. Richard Jed Wyatt, Chief, Neuropsychiatry Branch, IRP, NIMH; Dr. Craig N. Karson, Staff Psychiatrist, NPB, IRP, NIMH; Dr. William J. Freed, Chief, Preclinical Neurosciences Section, NPB, IRP, NIMH; Dr. Karen F. Berman, Staff Psychiatrist, NPB, IRP, NIMH; Dr. Esa Korpi, State Alcohol Monopoly, Helsinki, Finland; Dr. Markku Linnoila, NIAAA, NIMH; Dr. T. Peter Bridge, NPB, IRP, NIMH; Dr. Farouk Karoum, Chemist, NPB, IRP, NIMH

COOPERATING UNITS *(if any)*

NIAAA, NIMH; State Alcohol Monopoly, Helsinki, Finland; Uniformed Services University Health Sciences; University of California, San Diego

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Clinical Brain Studies Section

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	1.50	.50

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.)*

Postmortem studies in psychiatric disorders antemies to be a useful method for testing hypotheses. New findings include the following: (1) Decreased tritiated clonidine binding in the locus coeruleus of schizophrenic patients; (2) Increased methionine-enkephalin in the substantia nigra of schizophrenic patients; (3) Increased serotonin (5-HT) and 5-hydroxy-indoleacetic acid (5-HIAA) in basal ganglia and occipital cortex, respectively in schizophrenic patients; and (4) Decreases in 5-HT and 5-HIAA in hypothalamus and nucleus accumbens, respectively in suicides.

PHS 6040 (Rev. 1/84)

GPO 904-917

Collaborators:

Dr. C. Raymond Lake, Uniformed Services University Health Sciences, Bethesda, Maryland
Dr. John Hong, Laboratory of Preclinical Pharmacology, IRP, NIMH
Dr. Michael Iadarola, Laboratory of Preclinical Pharmacology, IRP, NIMH
Dr. S. Govoni, Laboratory of Preclinical Pharmacology, IRP, NIMH
Dr. J. Christian Gillin, University of California, San Diego, La Jolla, California

Project Description:

Objectives: Neurochemical analyses of postmortem human brain tissue is an area of increasing interest to psychiatric research. Many groups are now concentrating on mapping central neuronal pathways or identifying biochemical abnormalities in neurological and psychiatric diseases. Our studies have focused primarily on neurochemical hypotheses in schizophrenia and suicide. In the schizophrenic syndrome our efforts have focused most on catecholamines, indoleamines, and neuropeptides. On suicide studies, we have concentrated on indoleamines, norepinephrine, and acetylcholine.

Methods Employed: Brains are collected seven days a week from the D.C. Medical Examiners office by the medical staff fellow and a research assistant. Patient and control brains are dissected by the medical staff fellow and a research assistant according to international criteria. Careful matching for postmortem interval and for freezer storage time is done in addition to the routine age, race, and gender matching. Another variable that may require matching is the time of year at death. Diagnosis of patients is performed independently by two psychiatists, using the newly developed Diagnostic Evaluation After Death criteria.

New Findings: Schizophrenia studies: Preliminary findings include (1) decreased tritiated clonidine binding in the locus coeruleus of schizophrenic patients using an autoradiographic approach; (2) increased methionine-enkephalin in the substantia nigra; and (3) increased serotonin in the putamen and globus pallidus and increased 5-hydroxyindoleacetic acid (5-HIAA) in the occipital cortex. In addition we have been able to demonstrate haloperidol in schizophrenic brain tissue as well as an unexpected haloperidol glucuroinde conjugate.
Suicide studies: No differences were found in left or right frontal cortex norepinephrine or 3-methoxy-4-hydroxyphenylglycol. A second negative finding occurred in several brain regions with regard to muscarinic binding. Finally, the only positive finding was decreased serotonin in the hypothalamus and decreased 5-HIAA in the nucleus accumbens of suicides.

Significance to Mental Health Research: Schizophrenia studies: Consistent with earlier findings, the major neurochemical changes in brains of patient with schizophrenia occur in the basal ganglia or ventral striatum i.e., increased dopamine receptors, increased nucleus accumbens norepinephrine, and decreased met-enkephalin in the caudate. One more recent studies suggest that serotonin in the basal ganglia is also effected. Moreover, it would appear that the neurons that project to these structures are also abnormal i.e., met-enkephalin in the substantia nigra and tritiated clonidine binding in the locus coeruleus.
Suicide studies: The changes in serotonin in the hypothalamus and nucleus accumbens are consistent with a large body of literature suggesting that there are serotonergic mechanisms involved in suicides.

Proposed Course of Project: (1) Further measures of catecholamines and peptide and their receptor in the basal ganglia and their limbic system. (2) Further studies of the projections to the basal ganglia and the limbic system including the dorsolateral frontal cortex and the anterior vermis of the cerebellum. (3) Further studies of serotonin (5-HT) and 5-HIAA and their 5-HT binding sites in the brains of suicides.

Publications:

Korpi, E.R., Kleinman, J.E., Costakos, D.T., Linnoila, M. and Wyatt, R.J.: Reduced haloperidol in the postmortem brains of haloperidol and treated patients. Psychiatry Res. 11:259-269, 1984.

Kleinman, J.E.: Catecholamines in the brains of schizophrenic patients. In Racagni, G., Paoletti, R. and Kielholz, P. (Eds.): Clinical Neuropharmacology, Vol. 7. New York, Raven Press, 1984, pp. 912-913.

Bracha, H.S. and Kleinman, J.E.: Post mortem findings in schizophrenia. In Lake, R. (Ed.): Psychiatric Clinics of North America - Psychopharmacology. September, 1984.

Bridge, T.P., Kleinman, J.E., Karoum, F. and Wyatt, R.J.: Postmortem central catecholamines and antemortem cognitive impairment in elderly schizophrenics and controls. Neuropsychobiology, in press.

Korpi, E.R., Kleinman, J.E., Goodman, S.I., Phillips, I., DeLisi, L.E., Linnoila, M. and Wyatt R.J.: Serotonin and 5-hydroxyindoleacetic acid concentrations in different brain regions of suicide victims: Comparison with chronic schizophrenic subjects with or without suicide as cause of death. Arch. Gen. Psychiatry, in press.

Kleinman, J.E., Reid, A., Lake, C.R. and Wyatt, R.J.: Studies of norepinephrine in schizophrenia. In Lake, C.R. and Ziegler, M.G. (Eds.): The Catecholamines in Psychiatric and Neurologic Disorders, Chapter 14. Baltimore, Maryland, Williams and Wilkins, in press.

Kleinman, J.E., Hong, J., Iadarola, M., Govoni, S. and Gillin, J.C.: Neuropeptides in human brains--postmortem studies. Prog. Neuro-Psychopharmacol., in press.

Kleinman, J.E., Karson, C.N., Weinberger, D.R., Freed, W.J., Berman, K.F. and Wyatt, R.J.: Eye-blinking and cerebral ventricular size in chronic schizophrenic patients. Am. J. Psychiatry, in press.

Kafka, M.S., Kleinman, J.E. and Wyatt, R.J.: Neurpharmacological and clinical evidence of dopaminergic control. Psychopharmacology, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02265-01 NPB

PERIOD COVERED

October 1, 1984 through September 30, 1985

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

Catecholamine Binding Site Changes in Human Post Mortem Tissue

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

Grant N. Ko, M.D., Senior Staff Fellow, NPB, NIMH

Dr. M.J. Kuhar, Dept. of Neuroscience Johns Hopkins University; Dr. Debra Niehoff, Division of Physiological and Biochemistry, NIMR, London, England; Dr. James Unnerstall, Dept. of Neuroscience, Johns Hopkins University; Dr. William Freed, Chief, Preclinical Neurosections Section, NPB, NIMH; Dr. Joel Kleinman, Chief, Clinical Brain Studies Section, NPB, NIMH; Dr. Esa R. Kopi, State Alcohol Monopoly, Helsinki, Finland; Dr. Llewellyn B. Bigelow, Clinical Director, William A. White Division, NIMH

COOPERATING UNITS *(if any)*

Department of Neuroscience, Johns Hopkins University School of Medicine; State Monopoly, Helsinki, Finland

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Clinical Brain Studies Section

INSTITUTE AND LOCATION

NIMH, Saint Elizbeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	0.7	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.)*

NIMH and Johns Hopkins University are investigating the changes in receptor numbers in response to the loss of neuronal input in experimental animals as and a potential explanation for previously reported aberations in cerebrospinal fluid and brains of patients with the schizophrenic syndrome. Utilizing tritium labeled ligands, the technique of in vitro autoradiography and computerized densitometry have been used to assess the changes in receptor numbers resulting from denervation in animals with subsequent reversal following successful transplantation of replacement tissue in a rat model for denervation supersensitivity. In humans, preliminary evidence suggests that norepinephrine containing cell bodies at the locus coeruleus have fewer binding sites for NE feedback inhibition than comparable normals and suicide victims. This later finding is in keeping with previous reports of increased NE in brain regions post mortem of schizophrenic patients. The nature of this finding, whether it be a neuroleptic effect or an important clue to the pathophysiology of at least a subtype of the schizophrenic syndrome has yet to be determined.

PHS 6040 (Rev. 1/84)

GPO 904-917

Collaborators:

Dr. Richard Jed Wyatt, Chief, Neuropsychiatry Branch, NIMH

Project Descriptions:

Objectives: Recent reports have indicated that norepinephrine (NE) is elevated in the cerebrospinal fluid of schizophrenic patients as well as in the post mortem brains of these patients compared with control subjects. One explanation for this finding might be that autoregulatory receptors responsible for the inhibition of NE cell activity might be less sensitive than those of normal controls. Indeed, some studies of platelets and others utilizing pharmacologic challenge with drugs which exert their effect via these receptors suggest that this is the case. The purpose of the present study is to visualize directly post mortem specimen binding sites for feedback inhibition of the NE cell bodies in schizophrenic patients, suicide victims and normal controls. A comparison of the receptor density in these three groups would clarify this possible explanation of the previously reported increase in NE activity in those regions that receive NE innervation.

In addition to the recent reports of NE increases in schizophrenia, this project addresses the possibility that the number of these feedback receptors is increased in depression, in that recent reports have implicated that the mechanism of action of antidepressants is through decreasing the sensitivity of these feedback receptors. Some reports utilizing pharmacologic challenges have suggested that these feedback receptors are subsensitive in depressed patients.

This project has begun with a proven animal model, namely denervation supersensitivity, in its exploration of the applicability of in vitro autoradiography for the detection of receptor number changes. The association of increased receptor numbers and their subsequent reduction to normal numbers with the behavioral manifestations of denervation and subsequent replacement of nervous tissue through surgical grafting of new tissue supports the feasibility of autoradiogaphy for detection of receptor number changes in psychiatric diseases.

Methods Employed: Post mortem specimens have been obtained from the office of the medical examiner, District of Columbia. The brainstems from five schizophrenic patients (mean age at time of death=43 years, mean post mortem interval=19 hours), six normal controls (52 years, 20 hours) and seven suicide victims (45 years, 21 hours) were obtained. Coronal pontine slices through the locus coeruleus were prepared for autoradiography as described in published works from this project's collaborators.

From hospital records of the deceased, the Diagnostic Evaluation After Death (DEAD) criteria for diagnosis, developed at the NIMH, were applied to determine grouping of samples. One specimen from each of the three groups was prepared together for assessment of receptor numbers to minimize interassay variability.

The animals utilized in this project had lesions of the substantia nigra and subsequent transplants of fetal SN and sham tissues. Spiroperidol autoradiography was performed on sections of striatal tissue.

In addition, a pilot study utilizing clonidine, a drug used to treat hypertension which acts at feedback inhibitor sites of NE cells, for the treatment of schizophrenia was begun. A double blind, placebo controlled treatment design was utilized and a protocol for human subject investigation was approved by the FDA and the Saint Elizabeths Hospital Institutional Review Board for this study. Since neuroleptics are used in this pilot study, and are known to interact with NE receptors, neuroleptic concentrations are being ascertained in these

patients. Radioreceptor assay and high performance liquid chromatography assay of these concentrations have been described.

Major Findings: The mean SEM receptor number concentrations for the three groups was 190 ± 10 femtomoles for the schizophrenic specimens, 887 ± 315 for the normal controls, and 617 ± 270 for the suicide victims. There was not significant difference in the mean age or post mortem interval between groups. This preliminary finding is consistent with the hypothesis that autoregulatory feedback receptors on the locus coeruleus for schizophrenic patients are decreased in number. The importance of this finding for the pathogenesis of the schizophrenic syndrome has yet to be elucidated. The most conservative explanation for this finding is that chronic neuroleptic treatment induces this change. Suicide victims were not different from controls. If suicide is a result of depression, then this finding does not support alterations of alpha 2 receptor density as an explanation for depression.

The SN lesioned animals used for the first part of this project that showed greater than 20% reduction in rotational behavior following surgical transplantation of replacement tissue had a right-left difference of 0.42 fmol/mg protein striatal binding sites, whereas those animals with less than 20% reduction in rotational behavior had a right-left difference of 5.5 fmol/mg protein. This is the first study to utilize autoradiography to demonstrate that increases in receptor numbers accompanies denervation, and that return to normal receptor numbers can accompany successful transplantation of catecholamine replacement tissue. This supports the utility of in vitro autoradiography for exploration of receptor changes associated with neuropsychiatric conditions.

New Findings: Two of the suicide specimens had receptor concentrations in the range as low as the schizophrenic group. Suicide has been reported to occur frequently among schizophrenic patients, and there may be some discrepancy in post hoc diagnostic procedures even utilizing DEAD criteria. Attempts to find sources of autopsy material with DSM-III antemortem diagnoses are being explored.

Significance to Mental Health Research: This project is an attempt to identify a specific receptor change associated with the schizophrenic process and suicide victims. Further, this project endeavors to find a substitute medication, based on a rationale derived from published scientific literature, in order to obviate the risks associated with currently used antipsychotic medication. There exist in the literature reports of the efficacy of clonidine in treating psychosis. Our experience to date is that clonidine is of equivocal utility.

Proposed Course of the Project: We intend to prepare more post mortem specimens for autoradiography and bring the total number to 10 specimens per group. We intend to evaluate the effect of clonidine as an adjunctive medication to neuroleptics in 10 patients, and to explore the effect of clonidine and neuroleptics on NE metabolite concentrations in live patients.

Publications:

Korpi, E.R., Ko, G.N., Phelps, B.H. and Wyatt, R.J.: Possible interference by the reduced haloperidol metabolite with the radioimmuno- and radioreceptor assay of blood haloperidol. J. Clin. Psychopharmacol., 4:332-335, 1984.

Ko, G.N., Korpi, E.R., Freed, W.J., Zalcman, S.J. and Bigelow, L.B.: Effect of valproic acid on behavior and plasma amino acid concentrations in chronic schizophrenic patients. Biol. Psychiatry 20:199-228, 1985.

Ko, G.N., Korpi, E.R., and Linnoila, M.: On the clinical relevance and methods of quantification of plasma concentrations of neuroleptics. J. Clin. Psychopharmacol., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02266-01 NPB

PERIOD COVERED

October 1, 1984 through September 30, 1985

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

Quantitative Assessment of Motor Function in Schizophrenia

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

James B. Lohr, M.D., Medical Staff Fellow, NPB, IRP, NIMH

Dr. Dilip V. Jeste, Medical Officer, NPB, IRP, NIMH; Dr. Jerome Sanes, National Institute of Neurological and Communicative Disorders and Stroke, NIH; Dr. Richard Jed Wyatt, Chief, Neuropsychiatry Branch, IRP, NIMH

COOPERATING UNITS *(if any)*

NINCDS, NIH

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.20	.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.)*

Schizophrenic patients (both on and off neuroleptics) and non-schizophrenic control patients are being evaluated using an apparatus designed to measure reaction time, movement time, and the pattern of muscle activity during rapid movement.

With this technique specific aspects of basal ganglia function in schizophrenia will be assessed, as well as motor disturbances related to neuroleptic medication.

PHS 6040 (Rev. 1/84)

GPO 904-917

Project Description:

Objectives: It is widely agree that disorders of movement may accompany schizophrenia, including tremor, akinesia, choreoathetosis and others, and that these movement disorders resemble those which occur in a variety of neurological disorders involving the basal ganglia, such as Huntington's and Parkinson's diseases. What is not clear is whether the abnormalities seen in schizophrenic patients are only secondary effects of the medications used to treat them, or whether they are primary clinical aspects of the schizophrenic syndrome. Furthermore, it is unclear if the parkinsonian signs observed in schizophrenia are really due to the same underlying pathophysiological mechanisms as in the neurological diseases.

There is a body of evidence implicating basal ganglia dysfunction in schizophrenia proper, apart from any dysfunction caused by neuroleptic drugs. Most of this evidence is indirect. For example, it has been found that the basal ganglia were the most frequently observed sites of damage in patients with neurological diseases whose symptoms resembled schizophrenia. Other evidence includes the similarity of the early stages of Huntington's disease in some patients to schizophrenia, the higher than normal frequency of parkinsonism in schizophrenics observed before the neuroleptic era, and the possibility that the basal ganglia have a role in attentional and affective processes that are disturbed in schizophrenia. More direct evidence includes the finding of abnormal cellular structure in the striatum of schizophrenics.

In order to help clarify the issue of basal ganglia involvement in the schizophrenic process, we are studying a sample of schizophrenic patients both on and off medications, using a system designed to assess quantitatively aspects of the motor system thought to be related to basal ganglia function. This apparatus has been developed by Dr. Jerome Sanes of the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) and has already been employed in the motor assessment of a variety of neuropsychiatric illnesses. In essence, the apparatus measures reaction time, movement time, and the electromyographic architecture of rapid movements of the arm. All three of these have been observed to be abnormal in Parkinson's disease and are readily quantifiable.

This study will attempt to provide preliminary answers to several questions: (1) Do schizophrenic patients on neuroleptics have abnormalities in reaction time (RT), movement time (MT) and fast movements? (2) How do non-schizophrenic psychiatric patients on neuroleptics perform on these three measures? (3) Do schizophrenics on neuroleptics perform like patients with Parkinson's disease on these three measures? and (4) Does performance on RT, MT, and fast movement of schizophrenics improve or normalize when they are taken off medications?

With these instruments we hope to gain further insight into the presence and nature of certain motor function abnormalities of schizophrenics in an attempt to help define the involvement of the basal ganglia. Furthermore, we hope to provide some quantitative techniques whereby certain motor side-effects of neuroleptics may be quantified.

Methods Employed: Subjects are 30 schizophrenic patients from Saint Elizabeths Hospital; 10 non-schizophrenic psychiatric patients on neuroleptic medications, 10 patients with Parkinson's disease, and 10 normal controls volunteers, all under 70 years of age. No schizophrenic or non-schizophrenic psychiatric patients with a family history of movement disorder is included.

Each subject is instructed to grasp a handle coupled to the axle of a low friction brushless DC torque motor (Aeroflex TQ 64 W-5). The handle can be rotated by wrist flexion or

extension, and the amount of handle movement is visually displayed on an oscilloscope screen. A small box appears on the screen and the subjects are asked to place a cursor, controlled by their wrist movement, into the box as soon as it appears on the screen. At the same time, surface EMG's are obtained from the flexor and extensor carpi ulnaris muscles. Five sets of 50 wrist movements are performed and analyzed per subject. The approximate testing time is one-half hour per subject.

Major Past Findings: Using this apparatus, it has been determined that the stereotyped triphasic EMG pattern appears to be a fundamental property of a central program underlying rapid movements. It is not yet known, however, if the central program is disrupted in schizophrenia.

New Findings: Data are currently being collected and analyzed.

Significance to Mental Health Research: The results of this study may contribute to our understanding of motor dysfunction in schizophrenia, and especially of extrapyramidal side-effects of neuroleptic medications. Such knowledge could aid in our treatment of extrapyramidal reactions.

Proposed Course of Project: Data collection and analyses should be finished in the next six to 12 months and results will be reported in next year's Annual Report.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02267-01 NPB

PERIOD COVERED

October 1, 1984 through September 30, 1985

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

Brain Electrical Activity Mapping in Neuropsychiatric Patients

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

John M. Morihisa, M.D., Chief, Clinical Neurophysiology Unit, NPB, IRP, NIMH

Dr. Richard Jed Wyatt, Chief, Neuropsychiatry Branch, IRP, NIMH; Dr. Daniel R. Weinberger, Chief, Section on Clinical Neuropsychiatry, NPB, IRP, NIMH; Dr. Frank H. Duffy, Chief, Developmental Neurophysiology, Harvard Medical School, Boston, MA.; Dr. Richard Coppola, Lab. of Psychology and Psychopathology, IRP, NIMH, Dr. Jack A. Grebb, Staff Fellow, NPB, IRP, NIMH

COOPERATING UNITS *(if any)*

Department of Neurology, Harvard Medical School; Department of Psychiatry, Columbia University College of Physicians and Surgeons

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Clinical Neuropsychiatry Section

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.70	1.20	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 research applying brain electrical activity mapping (BEAM) preliminary investigations have been completed for schizophrenic patients on and off medications, childhood autism with age matched controls and a pilot study of patients with Alzheimer's disease. In addition, a preliminary study that combined a structural research technique (computed tomography) and a functional measure (BEAM) was completed. Also data is presently being analyzed from a study specifically investigating the effects of haloperidol on the same schizophrenic subjects on and off medications. To date, results indicate that patients with schizophrenia exhibit increased delta activity compared to normal controls regardless of their medication state. Furthermore, this increased delta activity in topographically most prominent over the frontal lobes. Thus, a converging and topographically congruent body of findings have evolved to implicate frontal lobe dysfunction as a pathologic process important to our understanding of schizophrenia.

PHS 6040 (Rev. 1/84) GPO 904-917

Collaborators:

Dr. Monte S. Buchsbaum, Professor of Psychiatry, University of California, Irvine
Dr. Allan Mirsky, Laboratory of Psychology and Psychopathology, NIMH
Dr. Lynn E. DeLisi, Laboratory of Psychology and Psychopathology, NIMH
Dr. Craig N. Karson, Staff Psychiatrist, NPB, IRP, NIMH
Dr. Wallace B. Mendelson, Chief, Unit on Sleep Studies, CPB, NIMH
Dr. Henry Holcomb, Biological Psychiatry Branch, NIMH
J. Johnson, NIAAA, NIMH
Dr. Robert Post, Chief, Biological Psychiatry Branch, NIMH
Dr. William Carpenter, Director, Maryland Psychiatric Center, Baltimore, Md.
Dr. Robert Cohen, Staff Psychiatrist, Clinical Neuropharmacology Branch, NIMH
Dr. David Pickar, NSB, NIMH
Dr. R. Magolin, CC, NM, NIH
Dr. R. Kessler, CC, NIH
Dr. John Boronow, NSB, NIMH

Project Description:

Objectives: Almost from its introduction EEG abnormalities have been reported more commonly in patients with schizophrenia than in the general population. No consensus, however, exists as to what abnormalities are characteristic to this disorder nor which, if any, are important to our understanding of the schizophrenic process. In the last six years several computerized topographic techniques have been introduced to electrophysiological research. Since 1979 we have built a clinical neurophysiology unit that has the capability to apply two of these computer mapping techniques to the study of EEG and evoked potential abnormalities in patients with schizophrenia. Encouraged by preliminary findings in schizophrenia that fit well with some of the other brain imaging techniques, we have expanded our investigations to include patients with childhood autism, Alzheimer's disease and patients with unipolar affective illness treated with ECT. In addition, this unit has been used as a resource for the investigation of clinical cases of special interest, this has included patients with atypical seizure disorders, a patient with cortical deafness, several patients with multiple personality and a patient referred by Children's Hospial for abnormalities of delta activity. It is our overall goal to provide a resource for investigating neuropsychiatric populations using computerized topographic methods of measuring EEG and evoked potential data.

Methods Employed: Neuropsychiatric patients from the research wards of the Neuropsychiatry Branch, The Washington Children's Hospital and the research wards of the Department of Biological Psychiatry at the College of Physicians and Surgeons of Columbia University have been studied using the computerized topographic techniques of the Clinical Neurophysiology facility. In all studies except the project at Columbia each patient or control was studied in two resting states and for auditory and visual evoked potentials. In the Columbia study, patients with unipolar affective disorder were studied at specific times before, during and after they received ECT with recordings of their EEG activity. In addition, the electrical concommitants of electroconvulsive therapy were investigated. Patients fulfilled DSM III and Research Diagnostic Criteria and were compared to controls that were not significantly different for age, sex or handedness.

In addition to the study of schizophrenia, childhood autism, Alzheimer's disease and unipolar affective illness, three drug effect studies are being conducted. A study of the effects of haloperidol looks at the same ten schizophrenic patients during a standard dose of haloperidol (.4 mg/kg) and four weeks drug free has been completed. A study of the electrophyisological effects of vasopressin and veraparnil on patients with schizophrenia is presently in progress using double blind crossover protocol.

Findings: In a recent study we found correlations of electrophysiological activity in the evoked potentials of schizophrenic patients that were associated with measures of structural abnormality. This study used a structural measure, ventricular brain ratio to look at a group of drug-free schizophrenic patients and a group of medicated schizophrenic patients. This study highlighted an electrophysiological difference between schizophrenic patients with normal ventricular size versus patients with enlarged ventricles. In addition, the study also suggests that this relationship between anatomic structure and electrophysiological function was not attributable to a medication effect.

An extension of previous work in schizophrenia studied additional patients to look specifically at the effects of haloperidol on a group of schizophrenic patients. This study had found a wide range of electrophysiological response in patients with schizophrenia to a standard dose of haloperidol (.4 mg/kg).

Significance to Mental Health Research: The clinical neurophysiology unit has provided us an opportunity to use an electrophysiological measure of pathology in schizophrenia that complements other research techniques presently being applied. The use of these complementary approaches provide another avenue of investigation that is both non-invasive, of no known risks and that can examine cognitive phenomena that persist for milliseconds. The work thus far has provided neurophysiological evidence that supports a growing body of work that is elucidating the role of frontal lobe pathology in schizophrenia. Furthermore, this technique promises to provide a similar neurophysiological window on function for disease such as childhood autism, Alzheimer's disease and the effect of ECT on unipolar affective illness. Finally, this unit's approach may be particularly sensitive to the effects and possible mechanisms of action of drugs such as haloperidol, vasopressin and verapamil.

Proposed Course of Project: Initial studies have been completed but studies of larger test groups as well as different psychiatric disorders are necessary to better define the possible significance of these findings to the pathophysiology of these neuropsychiatric disorders.

Publications:

Morihisa, J.M., Duffy, F.H. and Wyatt, R.J.: Brain electrical activity mapping in psychiatry. In Morihisa, J. (Ed.): Brain Imaging in Psychiatry. Washington, D.C., American Psychiatric Press, 1984, pp. 77-93.

Morihisa, J.M.: Brain Imaging in Psychiatry. Washington, D.C., American Psychiatric Press, 1984.

Buchsbaum, M.S., Mirsky, A., DeLisi, L., 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 Res. 13:95-108, 1984.

Buchsbaum, M., DeLisi, L., Holcomb, H., Cappelletti, J., King, C., Johnson, J., Hazlett, E., Dowling-Zimmerman, S., Post, R., Morihisa, J., Carpenter, W., Cohen, R., Pickar, D., Weinberger, D., Margolin, R. and Kessler, R.: Anteroposterior gradient in cerebral glucose use in schizophrenia and affective disorders. Arch. Gen. Psychiatry 41:1159-1166, 1984.

Morihisa, J.M. and McAulty, G.B.: Structure and function: Brain electrial activity mapping (BEAM) and computerized axial tomography (CAT Scan) in schizophrenia. Biol. Psychiatry 20:3-19, 1985.

Morihisa, J.M., Duffy, F.H. and Wyatt, R.J.: Brain electrical activity mapping (BEAM) investigation of schizophrenia. In Buchsbaum, M., Usdin, E., Bunney, W. and Ingvar, D. (Eds.): Brain Imaging in Psychiatry and Neurology: PETT and Other Techniques. Pacific Grove, California, Baxwood/Synapse, in press.

Morihisa, J.M., Duffy, F.H. and Wyatt, R.J.: Event related potentials in schizophrenia using brain electrical activity mapping. J. Electroencephalo. Clin. Neuropsychiol., in press.

Morihisa, J.M.: Brain imaging techniques applied to electrophysiologic research in psychiatry. Psychiatr. Ann., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02268-01 NPB

PERIOD COVERED

October 1, 1984 through September 30, 1985

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

The Clinical Phenomenology 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, NPB, IRP, NIMH

Dr. E.A. Silberman, USUHS; Dr. Robert Post, Biological Psychiatry Branch, IRP, NIMH

COOPERATING UNITS *(if any)*

USUHS
Biological Psychiatry Branch, IRP, NIMH

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.75	.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 clinical syndrome of multiple personality disorder (MPD) is an unusual dissociative condition that has been poorly characterized. In an attempt to better delineate the clinical phenomenology of MPD, 100 recent cases were collected on a 386-item questionnaire completed by clinicians involved in the treatment of MPD patients. This study documents the existence of a clinical syndrome characterized by a core of depressive and dissociative symptoms and a childhood history of significant trauma, primarily child abuse.

PHS 6040 (Rev. 1/84) GPO 904-917

Project Description:

Objectives: Until the last decade, multiple personality disorder (MPD) has been considered to be an extremely rare dissociative condition. With the inclusion of MPD in the DSM-III (1980), there has been a dramatic increase in the numbers of reported cases. The purpose of this project is to survey current cases in the United States and Canada to determine the clinical presentation of MPD cases, the past psychiatric, medical, childhood and family history of these individuals, the phenomenology of the patient's alternate personality system and responses of the patient to standard therapeutic interventions.

Methods Employed: A 386-item questionnaire was developed, tested and refined. The questionnaire form is designed to collect detailed information on a single case. The questionnaire covers seven general areas: information on the reporting clinician and demographic data on the patient; presenting symptoms; past psychiatric, family and childhood history; method of diagnosis of MPD; alternate personality characteristics; treatment techniques; and therapeutic outcome of the case. Three basic types of questions are used throughout the questionnaire: symptom checklists, multiple choice questions (with opportunity for additional filled-in choices) and rating scales. Standardized scales are used wherever applicable.

Case sampling consisted of distributing the questionnaire to clinicians known to be interested in the disorder. A national sampling representing 48 states and all Canadian provinces were obtained. The return rate was 40%. Each clinican receiving the questionnaire was asked to report on a single patient, currently or recently in treatment with that clinician, who met DSM-III criteria. All questionnaires were key-entered into the NIH computer system and cases were cross-checked against each other by initials and birthdate to determine if any patient was collected more than once. Behavior and symptom arrays, created by grouping related items, were used in parametric data analysis.

Major Past Findings: The patient population was found to be predominantely female (92%) with a mean age at the time of questionnaire administration of 35.8 (range 11-56) years. Patients were poly-symptomatic on initial clinical presentation (mean number of symptoms 18.5) with psychatric symptoms of depression (depressed mood, mood swings, insomnia, sexual dysfunction, suicidality) being the most common clinical presentation. Somatic symptoms of headache, and GI disturbances were also commonly reported. The average number of alternate personalities was 13.3 (median 8) with number of types of alternates being very commonly reported across cases. These included child personalities, personalities who persecuted the individual (e.g., by self-mutilation), personalities who had continous memory for the life history of the individual, cross-gender alternates and personalities who were analgesic for painful stimuli. In every case, alternate personality-specific psychopathology was reported and responsible for the individual's life difficulties.

A common history trauma was found in 97% of the reported cases. In most cases, 85%, this was due to sexual abuse. Incest accounted for 69% of the cases of abuse. Physical abuse was also commonly reported (75%) with a large percentage of cases having a history of both types of abuse. The witnessing in childhood of the violent death of a parent, sibling or playmate was also a surprisingly common finding (45%).

New Findings: Data from this study are being analyzed for variables related to treatment response. The patients, on the average, had received four prior psychiatric diagnoses and

many of the standard psychiatric treatments, including most classes of medication, ECT and a variety of psychotherapy and milieu therapies.

Significance to Mental Health Research: This project represents the first attempt to conduct a large scale sampling of independent cases of multiple personality disorder. The results suggest that there is a core syndrome with many cases having a highly similar clinical phenomenology. The data suggest that these patients were often mistakenly misdiagnosed in the past. Better characterization of the disorder will permit improved recognition and more appropriate treatment.

Proposed Course of the Project: The current sampling phase of this project has been completed. Further data analysis is planned concerning variables related to treatment and outcome. A follow-up sampling of collected cases is being considered.

Publications:

Putnam, F.W., Guroff, J.J., Silberman, E.K., Barban, L. and Post, R.M.: The clinical phenomenology of multiple personality disorder: 100 recent cases. J. Clin. Psychiatry, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 MH 02269-01 NPB

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Development, Reliability and Validity of a Dissociation Scale

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Frank W. Putnam, M.D., Staff Psychiatrist, NPB, IRP, NIMH

Eve Bernstein, Department of Psychology, American University

COOPERATING UNITS *(if any)*

Department of Psychiatry, American University

LAB/BRANCH
Neuropsychiatry Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.20	.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.)*
This project concerns the ongoing development, reliability and validity of a psychometric instrument to measure the degree and type of dissociative psychopathology in an individual.

PHS 6040 (Rev. 1/84) GPO 904-917

Project Description:

Objectives: The phenomenon of dissociation is a psychophysiological process that may contribute to the psychopathology of a wide range of psychiatric disorders. The recognition and measurement of dissociative phenomena has been hampered by the lack of reliable and valid psychometric instruments. In collaboration with Eve Bernstein, doctoral student in the Department of Psychiatry, American University, we have been developing and testing a self-administered, 28-item questionnaire/scale to measure dissociative phenomena.

Methods Employed: Reliability testing has included both test-restest and split half measures with a test-retest interval of four weeks. Validity testing consists of comparing individual items and overall scores across normal controls and schizophrenic, agoraphobic, phobic/anxious, eating disorder, post-traumatic stress, major affective, obsessive-compulsive, alcoholic and multiple personality disorder patient groups.

New Findings: For normal control subjects the test-retest reliability coefficient (Spearman) was $r=.71$ ($p < 0.05$, $n=27$). Split-half reliability coefficients were $r=.67$ ($p < 0.007$, $n=27$) for normal controls, $r=.91$ ($p < 0.003$, $n=15$ for schizophrenic patients, $r=.86$ ($p < 0.001$, $n=27$) for agoraphobic patients and $r=.91$ ($p < 0.0004$, $n=13$) for multiple personality patients. Current median scores of subjects by group yielded a Chi-square of 45.42 ($p < 0.0001$, $df=3$). Pair-wise comparisons of groups yields signficiant differences across all groups tested to date. Age, a variable known to be significantly related to the incidents of depersonalization, was non-significant across all groups.

Significance to Mental Health Research: Dissociation is thought to play a major role in the persistence of certain types of symptoms secondary to traumatic experiences, e.g., flashbacks, intrusive thoughts and images. This project seeks to develop a reliable and valid instrument to survey the degree of dissociative phenomena in a wide range of psychiatric disorders. A better understanding of athe contributions of the dissociative process to psychopathology may be useful in the treatment of refractory symptoms.

Proposed Course of Project: We plan to continue validity and reliability testing of this instrument over the next reporting year.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02270-01 NPB

PERIOD COVERED

October 1, 1984 through September 30, 1985

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

The Psychophysiology of Multiple Personality

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

Frank W. Putnam, M.D., Staff Psychiatrist, NPB, IRP, NIMH

Dr. Robert M. Post, Biological Psychiatry Branch, IRP, NIMH; Dr. Karen Berman, Staff Psychiatrist, NPB, NIMH; Dr. John Morihisa, Staff Psychiatrist, NPB, NIMH; Dr. Daniel Weinberger, Chief, Clinical Neuropsychiatry Section, NPB, NIMH; Dr. Theodore Zahn, Laboratory of Psychology and Psychopathology, NIMH; Dr. Richard Coppola, Laboratory of Psychology and Psychopathology, NIMH

COOPERATING UNITS *(if any)*

Laboratory of Psychology and Psychopathology, IRP, NIMH

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.80	.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.)*

This work seeks to measure reported physiological differences that may exist among the alternate personalities of individuals suffering from multiple personality disorder (MPD). Alternate personalities of MPD subjects are measured on visual and auditory evoked potentials, spontaneous EEG, cerebral blood flow and autonomic nervous system measured such as galvanic skin response, skin temperature, pulse and respiration.

PHS 6040 (Rev. 1/84)

GPO 904-917

Project Description:

Objectives: This project seeks to ascertain and measure any psychophysiological differences that may exist among the alternate personalities of individuals suffering from multiple personality disorder (MPD).

Method Employed: A variety of psychophysiological methods have been used in this study. These included averaged evoked potentials elicited by light and sound in collaboration with Drs. R. Copola (Lab. of Psychol, IRP) and J. Morihisa (NPB, IRP). Galvanic skin response, respiration and skin temperature is measured in collaboration with Dr. T. Zahn, (Lab. of Psychol) and cerebral blood flow in collaboration with Dr. D. Weinberger and K. Berman (NPB, IRP).

Past Findings: Studies from this project indicate that MPD patients can produce changes in visually evoked potentials that can not be duplicated by simulating normal control subjects.

New Findings: Analysis of data from "the switch study", a systematic look at the period before, during and after a new personality appears, suggests that there is a significant order effect, in that the alternate personality who is active prior to the switch to the new personality sets a physiological background against which the new personality expresses itself.

Significance to Mental Health Research: The demontration of state specific psychophysiological responses may permit a more extensive investigation of the role of personality factors in psychosomatic disorders. Multiple personality disorder subjects provide a unique window through which to manipulate state-specific phenomona.

Proposed Course of Project: A recent amendment to the protocol has been submitted that would allow the investigator to look at immune system changes associated with switches in alternate personalities. Such pilot studies are warranted by the frequent reports of alternate personality-specific allergic reactions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 02271-01 NPB

PERIOD COVERED

October 1, 1984 through September 30, 1985

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

Fenfluramine and Chronic Schizophrenia

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

David Shore, M.D., Staff Psychiatrist, NPB, IRP, NIMH and Richard Jed Wyatt, M.D., Chief, Neuropsychiatry Branch, IRP, NIMH

Dr. Llewellyn B. Bigelow, Director of William A. White Division, Saint Elizabeths Hospital, NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Section on Aging

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.75	.75	

CHECK APPROPRIATE BOX(ES)

- [X] (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 study of fenfluramine was conducted among chronic schizophrenic patients. Fenfluramine tended to improve activation and global BPRS ratings, while worsening BPRS negative symptoms. Blood serotonin concentrations for each subject declined by at least 50% on active fenfluramine.

PHS 6040 (Rev. 1/84)

GPO 904-917

Objectives: Four studies of blood serotonin in chronic schizophrenic patients have shown elevated concentrations in such patients. Research has shown results "similar to the high serotonin levels found in the blood of children suffering from early infantile autism, a disease that is considered by some to belong to the schizophrenic nosological group." Autistic children often have cortical atrophy, neurologic and cognitive abnormalities, high blood serotonin concentrations, and poor treatment responses. In both groups of patients, the cause is unknown, the prognosis is poor, and no specific histopathological abnormalities have been discovered.

In 1982, researchers reported positive therapeutic effects of fenfluramine on the behavior and cognitive test performance of three autistic children. Fenfluramine is an appetite-reducing (nonstimulant) drug approved for use in the treatment of obesity. In animal studies, the drug produced a long-lasting reversible decrease in brain serotonin. In man, the major metabolite of serotonin (5-hydroxy-indoleacetic acid; 5-HIAA) was reduced in the cerebrospinal fluid following 8 days of fenfluramine administration. It was also reported that when fenfluramine lowered blood serotonin concentrations in the autistic children studied, improved behavior and cognition were noted by blind raters.

Methods Employed: We measured whole blood serotonin concentrations and psychiatric symptoms (using the BPRS) during a double-blind, placebo-controlled study of fenfluramine in patients with chronic schizophrenia. We also determined correlations of blood serotonin concentrations with age, platelet number, and ventricle to brain ratio (VBR) on CT scans.

Eight chronic schizophrenic patients agreed to participate in the study. The protocol was approved by the Saint Elizabeths Hospital Institutional Review Board, and all patients gave written informed consent. Patients were given placebo for at least 3 weeks, followed by active fenfluramine for 7-8 weeks, and then placebo again for 7-8 weeks (ABA design). Throughout the trial, daily blind BPRS ratings were carried out by trained raters on the wards.

Findings: All eight patients completed the protocol. There were no significant drug effects on BPRS subscales, but there were several interesting trends. When the 3-week periods at the end of the fenfluramine and the two placebo periods were compared, fenfluramine trended to improve activation and global BPRS ratings, while worsening BPRS negative symptoms. Some patients appeared better able to express themselves coherently on fenfluramine, but most showed no clinically significant change. Two patients who appeared to clinically benefit from fenfluramine were given a second blind trial of the drug (ABAB design), but the improvements noted could not be replicated.

Blood serotonin concentrations for each subject declined by at least 50% on active fenfluramine. The mean serotonin concentration after 1 month of active drug was 35% of pretreatment levels, a statistically significant difference (matched pair $t=7.25$, $p=0.0004$). Serotonin concentrations typically were at their lowest levels after 3 weeks of active fenfluramine and returned to their pretreatment levels about 3 weeks after placebo substitution.

We found no significant correlation between pretreatment blood serotonin concentrations and VBR ratios derived from the patients' CT scans. There were no significant correlations

between any BPRS subscale scores and blood sertonin concentrations during placebo or active fenfluramine administration.

In summary, we have been unable to show significant benefits using fenfluramine in the treatment of chronic, treatment-resistant schizophrenic patients on fixed neuroleptic doses. Most patients experienced sedation, which prevented us from using th maximum approved dosage (120 mg/day). The trend toward improvement in the BPRS global and activation subscales was likely due to the sedative effects of the drug. The trend toward worsening of the BPRS negative symptom subscale during fenfluramine treatment is also consistent with such an interpretation.

Significance to Mental Heath Reserach: Chronic schizophrenic patients occupy a large percentage of mental hospital beds in the U.S. and some are not helped by current treatments enough to function independently. Any treatment that could help such treatment-resistant patients to function better would be of great benefit to psychiatry. Fenfluramine has been considered potentially useful for such patients, but this double blind study has failed to demonstrate any significant benefits.

Proposed Course of Project: Completed and terminated.

Publications:

Shore, D., Bigelow, L.B. and Wyatt, R.J.: Fenfluramine treatment in chronic schizophrenia. Biol. Psychiatry, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02272-01 NPB

PERIOD COVERED

October 1, 1984 through September 30,1985

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

Sodium Fluoride Treatment of Alzheimer's Disease (AD)

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

David Shore, M.D., Staff Psychiatrist, NPB, IRP, NIMH and Richard Jed Wyatt, M.D., Chief, Neuropsychiatry Branch, IRP, NIMH

Stewart M. Sprague, Michigan State University; Dr. G.H. Mayor, Michigan State Univeristy; Dr. R.I. Henkin, Georgetown University; N.R Nelson, Georgetown University; Dr. Carol Overman, Speech Pathology and Audiology Department, Saint Elizabeths Hospital; Mark Tecco, George Washington University

COOPERATING UNITS *(if any)*

Speech Pathology and Audiology Department, Rehabilitation Medicine Division, Saint Elizabeths Hospital, Washington, D.C.; Michigan State University, East Lansing, Michigan; Georgetown University, Washington, D.C.; George Washington University, Washington, D.C.

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Section on Aging

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.20	.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.)*

Six patients completed a two-year double-blind, placebo-controlled crossover design study of the effects of sodium fluoride on the course of Alzheimer's disease (AD). Patients were screened and only those meeting DSM III criteria for Primary Degenerative Dementia (senile or presenile onset) were entered into the study. Other inclusion/exclusion criteria are described in an accompanying publication. Patients were given a battery of cognitive, speech, and memory tests every three months while taking either 90 mg of sodium fluoride q hs (in controlled-release capsules) or placebo capsules similar in appearance. The rationale for this pilot study is based on the aluminum hypothesis of AD.

PHS 6040 (Rev. 1/84) GPO 904-917

Project Description:

Objectives: It has been shown that "senile plaques" and "paired-helical filaments" (PHFs) are neuropathological hallmarks of AD, and the degree of dementia correlates highly with the number of plaques and PHF-bearing neurons. Aluminum (Al) accumulations have been found in the nuclei of neurons containing these PHFs and there is concern that this metal may be a pathogenic factor in AD.

As early as 1885, Al was shown to have neurotoxic properties in animals. In 1965, Al was shown to produce a type of neurofibrillary degeneration in cats and rabbits which in some ways resembles that seen in AD patients' brains. Despite the similarities, the filamentous hyperplasia seen in Al-injected animals (and neuroblastoma cells cultured in a high Al media is ultrastructurally different from the PHFs seen in the brains of AD patients. It has been reported that Al injected into the CSF of certain animal species rapidly accumulates on the nuclear chromatin, with memory and learning impairments seen approximately 1 week later, when neurons show degenerative changes and neurofilament accumulation. Seizures and death typically occur 2 to 3 weeks after these changes appear.

Given the possibility that AD patients may be "vulnerable" to the low concentrations of Al normally present in the body, several research groups have considered ways to prevent Al from binding to nuclear chromatin in the brain. Because Al is tightly bound to DNA, and chelators and complexing agents do not easily cross the blood-brain barrier, removing Al directly from neuronal nuclei would be difficult. Agents that bind aluminum, however, might facilitate its excretion from the body and prevent further Al accumulation in neuronal nuclei. If Al has a genuine role in AD pathogenesis, then complexing and removal of Al might slow or halt the progression of this dementia. Methodological considerations involved in such a project have recently been published elsewhere.

Methods Employed: In rats, Fluoride (F) reportedly decreases Al absorption and increases the urinary excretion of Al. Given chronically (for 40 days), F led to a decrease in bone Al content in rats. One research group given a solution of F (with meals) to AD patients describes a possible "plateau" of symptoms while on F.

In animal studies done in our laboratory, we have tested the ability of F to prevent Al accumulations in rabbit brains. Animals pretested for 9 days with 3 mg of F/kg body wt./day had lower Al concentrations in some brain regions 3 weeks after intrathecal Al injections. Because of the small number of experimental and control rabbits and the variability of neuropathological changes, we must interpret such results with caution.

Findings: Several patients entered the protocol but were unable to continue due to progression of their illness to the point where they were untestable and/or unable to travel to the test site. Other patients were evaluated (and two were entered into the protocol) but found not to have the clinical course (during placebo administration) required to confirm the diagnosis of AD. No subject suffered any adverse effects of participation in the protocol. Analysis of test results demonstrated no significant effects of sodium fluoride on the symptoms and clinical deterioration of patients with AD.

Significance to Mental Health Research: Alzheimer's disease is the most common dementia of later life, and it produces a progressive deterioration in mental functioning that eventually requires total care of the patient. This study attempted to slow or halt the progression of dementia, which could decrease the disability caused by AD. Since sodium

fluoride and use of "chealtors" have been advocated for AD patients, we believe that this double blind trial was important to determine whether such treatments are likely to be effective.

Proposed Course of Project: Completed and terminated.

Publications:

Shore, D., Henkin, R.I., Nelson, N.R., Agarwal, R.P. and Wyatt, R.J.: Hair and serum copper, zinc, calcium, and magnesium concentrations in Alzheimer-type dementia. J. Am. Geriatr. Soc., 32:892-895, 1984.

Shore, D., Overman, C.A. and Wyatt, R.J.: More on Alzheimer's disease diagnosis. J. Clinical Psychiatry 45:282, 2984.

Shore, D., Sprague, S.M., Mayor, G.H., et al.: Aluminum-fluoride conplexes: Preclinical studies. In Liss, L. (Ed.): Clinical Implications of Aluminum Neurotoxicity. Pathotox Publications, in press.

Shore, D., Overman, C.A., Tecco, M. and Wyatt, R.J.: Longitudinal study of the course of Alzheimer's disease. In Jeste, D.V. (Ed.): Current Perspectives on Dementias. Washington, D.C., American Psychiatric Press, Inc., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 02273-01 NPB

PERIOD COVERED

October 1, 1984 through September 30, 1985

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

White House Cases: Predictors of Future Violence

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

David Shore, M.D., Staff Psychiatrist, NPB, IRP, NIMH

C. Richard Filson, Ed.D., Richardson Division, SEH; Kenneth Baker, Behavioral Research Section, U.S. Secret Service; Dr. Charles Kinderman, Bureau of Justice Statistics, U.S. Department of Justice; Ken Candell, Uniform Crime Reporting Division; William Garvie, Identification Division, FBI

COOPERATING UNITS *(if any)*

Richardson Division, Saint Elizabeths Hospital, Washington, D.C.; Behavioral Research Section, Intelligence Division, U.S. Secret Service; Bureau of Justice Statistics, U.S. Department of Justice; Uniform Crime Reporting Division and Identification Division, FBI

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Section on Aging

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.80	.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.)*

A series of studies of White House Cases (WHCs) is being conducted. Data from the FBI regarding age and gender specific rates of arrest for various crimes in the general population are being analyzed.

PHS 6040 (Rev. 1/84) GPO 904-917

Project Description:

Objectives: Delusional visitors to the White House or other government offices (often seeking a personal audience with the President) are interviewed by the Secret Service and then sent to Saint Elizabeths Hospital if they are considered mentally ill and potentially dangerous to themselves or others. A review of the demographic characteristics and diagnoses of 328 of these "White House Cases" treated at the hospital between 1970 and mid-1974 showed that these patients were most commonly unmarried, white, and male, and most had a diagnosis of paranoid schizophrenia. Although 22% of this group have threatened some prominent political figure, to date none of this study's patients has attempted to assassinate any such government official.

Methods Employed: We are now anlayzing data based on a review of the WHCs' detailed national arrest records. We are comparing the WHCs arrested for violent crimes (murder and assault) after their index hospital discharge with those having exclusively non-violent arrests and those having no arrests during follow-up.

Findings: Those with violent crime arrests after hospital discharge were more likely to be male and to have a history of prior arrests and threats against some Secret Service protectee.

Significance to Mental Health Research: Mental health professionals, especially psychiatrists in acute care and inpatient settings, are frequently called upon to evaluate the "dangerousness" of mental patients. Decisions about hospital admission (particularly involuntary committment), discharge, transfer from locked units, passes, etc. may require consideration of the likelihood that a patient may harm another person as a result of the mental disorder. There are currently few reliable and valid criteria for making such assessments. By obtaining complete nationwide arrest records and comparing rates of violent crime arrests of WHCs with rates in the general population, we hope to discover whether paranoid schizophrenic men with a history of acting on delusions are more likely to have violent crime arrests. By review of WHCs' charts, we hope to identify clinical features associated with future violent crime arrest in such patients.

Proposed Course: We are gathering data from the FBI regarding age and gender specific rates of arrest for various crimes in the general population. We will then be able to determine whether the rate of arrests for violent crimes and for robbery are higher or lower for the WHCs. Since many of the WHCs had prior arrests, any excess in post-hospitalization arrests might be due to this factor, and we are also taking steps to control for such. The FBI Identification division is providing a stratified random sample of arrest records for the general population, to be matched for age, race and gender with those WHCs having prior arrests. In this way we can calculate rates of arrest for various crimes among WHCs and general population samples with similar prior (i.e., pre-1973) arrest records, to determine whether the WHCs' arrest rates differ from a general population sample controlled for age, race, gender, and prior arrests.

Another study will involve a blind chart review of the male WHCs' hospital charts to discover whether any specific symptoms or other factors noted in the records are predictive of future violence. Since psychiatrists are being called upon to predict the "dangerousness" of mental patients, it would be useful to know which factors are associated with increased or decreased risk of violent behavior in a group of mainly paranoid schizophrenic patients with a history of acting on their delusional beliefs. Charts of 224 male non-forensic WHCs

with valid arrest records will be reviewed by two trained raters during the Summer of 1985. We anticipate that funds for these blind student raters will be provided by a grant from the Behavioral Research Section, U.S. Secret Service Intelligence Division.

Publications:

Shore, D., Filson, R., Davis, T.S., et al.: White House Cases - Psychiatry and the U.S. Secret Service. Am. J. Psychiatry, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02274-01 NPB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Exploration of new methods for treatment of intractable epilepsy

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

Janice Stevens, M.D., Medical Officer, NPB, IRP, NIMH

Dr. William Freed, Chief, Section on Preclinical Neurosciences, NPB, IRP, NIMH; Janice Miller, B.A., Dr. Urmi Patel, Staff Fellow, NPB, IRP, NIMH; Dr. Mike Iadarola, Staff Fellow, LPP, IRP, NIMH; Dr. Renaud Beaurepaire, Visiting Associate, NPB, IRP, NIMH.

COOPERATING UNITS *(if any)*

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Section on Aging

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.75	.75	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 an attempt to devise more effective methods for treatment of epilepsy that is intractible to conventional, modern medical therapy or surgical intervention, we are exploring the feasibility of brain grafts of GABAergic brain tissue to effected brain areas in rat models of epilepsy. We have developed two satisfactory experimental epilepsy models in the laboratory, the audiogenic seizures in a genetically pre-disposed strain of rats and amygdala kindling. Successful grafts of fetal cerebellar tissue to adult rat ventricles has been accomplished.

PHS 6040 (Rev. 1/84)

GPO 904-917

Project Description:

ZO1 MH 02274-01 NPB

Objectives: The goal of this work is to establish standard models of epilepsy in the rat to utilize in a test of feasibility for brain graft of GABAergic cells. Approximately 80 percent of individuals with epilepsy can obtain satisfactory seizure control with proper anti-convulsant therapy. Another three to four percent can be controlled or even cured by surgical treatment in which lobectomy (usually temporal), partial brain resections, corpus colostomy or even hemispherectomy are employed. To address more effectively the problems posed by the remaining approximately 15 percent of the patients with epilepsy whose seizures remain intractable despite these efforts or who are inappropriate for surgical intervention, we become interested in attempting to increase inhibition of seizure spread in areas of the brain where gamma-aminobutyric acid (GABA) is known to inhibit generalized seizures, by grafting GABA rich brain cells to these key areas.

Methods and Findings: In order to accomplish this we first had to establish a reliable model of epilepsy and to demonstrate that we could grow GABAergic tissue in graft. Two models of epilepsy have been used: 1) rats genetically predisposed to audiogenic seizures (ASP rats); 2) rats in whom epileptogenic fosi were attempted with injections of ferric chloride or aluminum hydroxide in motor cortex and amygdala. The audiogenic rats have been bred in the laboratory. These animals show a norepinephrine deficiency in the inferior coliculus and seizures can be prevented by injections of norepinephrine in this region. Thus we first explored the possibility of grafting locus ceruleus cells to this region in several rats. This had no effect on the seizure threshold or intensity and calculation of the amount of norepinephrine released by such grafts indicated that insufficient norepinephrine would be released by at least an order of magnitude. Injection of GABA in the same site also prevents seizures in these animals but is very unstable. We experimented with injections in several sites in the midbrain of the GABA transaminase inhibitor gamma-vinyl GABA and found that seizures could be suppressed by injections of this agent in the midbrain reticular formation. We have confirmed these findings in chronically implanted animals.

At the same time we were testing the audiogenic animals with success, we have had less satisfactory results with the focal cortical model of epilepsy. Although a few seizures or twitches might be obtained during the first three or four days after the ferric chloride innoculations of cortex, these focal seizures have not been sufficiently persistent to use as a model for the projected grafting studies. Accordingly this spring we are turning to the kindling model for focal epilepsy in order to have both a focal and a generalized (audiogenic seizures) model to work with the grafting studies.

At the same time we have been working on methods for identifying cerebellar grafts and demonstrating successful growth of GABAergic cells, using a GAD stain. Although we have now successfully transplanted whole tissue and experimented with dissociated cells we have not yet succeeded in demonstrating viable GAD-producing cells in these grafts. Since GAD staining is obtained in Purkinje cells in vibratome sections of cerebellum in the adult rat, we are not yet certain whether our problems are technical in handling the fetal material (which must be prefixed in paraffin and cannot be handled in the vibratome sections successfully with adult tissue) or whether GAD is not yet present in the 15-18 day fetuses employed. This work is still in progress. No ASP rats

have yet been implanted with the cerebellar graft tissue. Hundreds of tests have been done stabilizing the ASP rat thresholds and testing of a variety of areas in the brain for seizure suppression with gamma-vinyl-GABA and saline controls. More than 40 rats were tested with the ferric chloride implants in various parts of the brain without obtaining a satisfactory seizure model. We are to commence studies with the kindling model this spring.

Significance: Epilepsy is one of the most prevalent and most disabling neuropsychiatric problems in the United States, and indeed in the world. Epilepsy affects approximately 0.5 percent of the U.S. population. It is largely young people who are most affected and 20 to 25 percent of those effected are severely and permanently handicapped despite enlightened use of the most modern therapeutic measures. New approaches to both prevention and treatment are urgently required for this disorder.

Proposed Course of Project: Our immediate plans for this project are as follows:

1. Work will continue with the transplant of fetal cerebellum to adult rats. We will continue to attempt to identify whether these grafts contain GABA elements using immunocytochemistry and radioimmunoassay methods. Using GABAergic drug infusions we are presently investigating the quantity of GABA required to suppress audiogenic and kindled seizures in the most favorable anatomical sites for placement of the GABAergic grafts. Having now these two robust epilepsy models and good evidence as to what drugs are effective and in what dosage and site, we will proceed to the grafts in the coming year.

When we have satisfactory evidence of growth and GAD production in these transplants, we will start implants in midbrain of audiogenic seizure-prone (ASP) rats.

2. Preparation of the kindling model of focal epilepsy for trial with grafts in basal forebrain.

3. Transplant of the grafts to focal seizure rats.

Publications:

Stevens, J.: Schizophrenia and the brain. Arch. Gen. Psychiatry, 41:816-817, 1984.

Stevens, J.R.: Epilepsy and psychosis: Neuropathologic studies of 6 cases. In Bolwis T. and Trimble, M. (Eds.): Epilepsy and Psychopathology. London, John Wiley & Sons, Ltd., in press.

Stevens, J.R.: All that spikes is not fits: Epilogue and update. In Trimble, M. and Reynolds, T. (Eds.): What is Epilepsy. London, Churchill Livingston, in press.

Stevens, J.R. and Lansbury, M.B.: Limbic system epilepsy and psychosis. Psychiatry Research, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02275-01 NPB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Search for Virus in Post Mortem Brain of Patients with Schizophrenia

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

Janice Stevens, M.D., Medical Officer, Neuropsychiatry Branch, IRP, NIMH

Charles Kaufmann, M.D.,Senior Staff Fellow, NPB, IRP, NIMH; Richard Ziegler; Ph.D., U. Minnesota; W.Y. Wang, M.D., Visiting Associate, NPB, IRP, NIMH; Ashley Haase, M.D., U. Minnesota; John Langloss, D.V.M., AFIP; Joseph Parisi, M.D., AFIP; Paul Albrecht, M.D., NIH; Robert Yolken, M.D., Johns Hopkins; Joel Kleinman, M.D., Chief, Section on Clinical Brain Studies, NPB, IRP, NIMH; David Asher, M.D., NINCDS; Joan Schwartz, Ph.D., Chemist, LPP, IRP, NIMH.

COOPERATING UNITS *(if any)*

Dept. of Virology and Microbiology, University of Minnesota
Armed Forces Institute of Pathology

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Section on Aging

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.75	.75	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.)*

Based on substantial evidence that an infectious agent or agents may play a role in the etiology of at least a subtype of schizophrenic illnesses, we are searching for evidence of an infectious or toxic substance in schizophrenic brains. Studies undertaken to date include immunocytochemical investigations for antigens to cytomeglavirus (CMV), Herpes simplex virus (HSV), varicella virus (VAV), rubella and mumps. Although sporadic cases have shown positive results with immunocytochemical studies, these have been inconsistent and rare. We have also undertaken in situ hybridization probes for CNV, and cultivation of schizophrenic and control brain specimens on cultures of human and non-human neural tissue. Using special stains for glia we have evaluated the brains of guinea pigs and primates previously innoculated with schizophrenic and control brain tissue.

PHS 6040 (Rev. 1/84) GPO 904-917

Cooperating Units:

Dept. of Pathology, NIH
Dept. of Neurology, Johns Hopkins School of Medicine
Dept. of Pediatrics, Johns Hopkins School of Medicine
University of Pennsylvania School of Medicine
University of Oregon School of Medicine
Stanford VA Hospital
Dept. of Pathology, UCLA Medical Center

Objectives: The purpose of this investigation is to use the most advanced technology to search for evidence of an infectious or toxic agent as a significant etiologic factor in schizophrenia or a subgroup of schizophrenic patients. This work was stimulated by evidence from a number of sources including epidemiologic, immunologic, geographic and neuropathologic studies compatible with an infectious etiology in this disorder. Highlights of the evidence include increased immunoglobulin in the cerebrospinal fluid of schizophrenic patients to specific viral agents (CMV, HSV, seasonal birth peaks of schizophrenic patients, uneven geographic distribution, abnormal response of lithocytes to specific mitogens, toxic effects of urine serum or cerebrospinal fluid of schizophrenic patients on animal behavior and tissue cultures, gross and histologic neuropathologic changes in the brains of individuals with schizophrenia. Previous attempts to identify antigens or a viral gnome from schizophrenic brains or passage of this disorder to animals or through cell cultures have generally been negative. There are, however, just enough positive results to require extension, replication and introduction of more advanced and sensitive methods. We are in an unusually favorable position to make such invetigations. An unusually rich collection of pathologic material from young schizophrenic patients most of whom died by suicide during an active phase of their illness are available. Matched controls from the same source, i.e., the Washington, DC Medical Examiner's Office, assure in so far as possible the simultaneous evaluation of both schizophrenic and non-schizophrenic tissue from a matched age group. This project seeks detection of a possible infectious agent in this neuropathologic material. This study has four parts.

A. Examination of post mortem tissue with immunocytochemical techniques.

B. In situ hybridization with viral genome probes.

C. Tissue culture: co-cultivation, "infection" and "superfection" with schizophrenic tissue.

D. Passive transfer to experimental animals.

Methods Employed and Major Findings:

1. Examination of post mortem tissue: This investigation was undertaken to search for evidence of viral infection or immunologic abnormality in the brains of patients with schizophrenia. The investigation commenced with immunocytochemical studies of fixed brain sections of basal ganglia, basal forebrain, brainstem and hypothalamus from the formalin fixed material in the Blackburn Laboratory collection of brains. Antibodies against cytomeglavirus (CMV) and herpes simplex virus 1 and 2 (HSV 1, 2) were employed. During the last year frozen specimens of hippocampus, hypothalamus, and amygdala from the frozen brain specimen bank have been used in similar studies. Antibodies to measles, mumps, varicella (VZV) and Epstein-Barr (EBV) virus have been added to the original panel of CMV and HSV antisera. This work was undertaken because of anti CMV lgG and lgM found in cerebrospinal fluid of schizophrenics. Specimens from a total of 24 brains from schizophrenic patients, six fixed and 18 from frozen material have now been studied. A special effort was made to study material from patients who had been ill for less than six years to maximize the possibility of finding antigen. Although initial results suggested antibodies to CMV (but never to HSV) in the fixed material, as more purified antibodies

including monoclonals were obtained, positive results were no longer consistently obtained. Results with the other antibodies gave moderately or inconsistently positive results in single cases against mumps and rubella but this occurred equally in controls and schizophrenic brains.

2. Genome Hybridization Studies: Frozen specimens from basal forebrain of schizophrenic and control brains were sent for genome hybridization studies with CMV and other viral probes. Thus far only CMV has been done on the first 10 cases and another 12 patients and 12 controls await investigation. No positive results have yet emerged.

3. Tissue Culture: Because the single probe method for a specific virus has yielded inconsistent results in our hands we have now turned to a more general "wider net" procedure in the search for an infectious agent significantly associated with schizophrenia. Our current efforts are directed towards "rescuing" latent virus by co-cultivation, utilizing tissue cultures of human neuronal tissue co-cultivated with fresh specimens from schizophrenic brains. Because the opportunity to obtain the latter is sporadic and unpredictable, we are also applying to the tissue cultures fresh spinal fluid from schizophrenic and control individuals and extracts of frozen brain specimens of selected brain regions from the large brain bank collection of schizophrenic and control brains.

Tissue culture of the established cell lines (human neuroblastoma, human medullablastoma, guinea pig hamster neuroblastoma and fetal dorsal root ganglia) are presently being maintained. In addition, guinea pig neuroblastoma hybrid allegedly containing dopamine receptors will be studied, both before and following addition of the schizophrenic material. All of these cultures are up and running at the present time so that we are prepared at any time of the day or night to undertake the co-cultivation when fresh schizophrenic brain material becomes available. In the interim application of frozen tissues continues in cultures.

4. Passive transfer experiments: Five years ago, 20 guinea pigs and 20 non-human primates were intracerebrally innoculated with homogenized brain from 10 schizophrenic patients and 10 neurologic controls. As these animals died or were sacrificed, brains were examined with conventional and ultramicroscopic techniques. Nothing was found. Because previous studies had shown gliosis to be the only relatively consistent change in schizophrenic brains, we reexamined the animal material using specific stains for fibillary gliosis. This we have done with the Holtzer and GFAP stains as well as stains for myelin and axis cylinders in all the material from the experimental animals. There was increased gliosis in four of the guinea pig brains and two of the primates. It was of interest that in the latter and in two of the four guinea pig brains, specimens were from the only early schizophrenic in the series of innoculated brains. It is of further great interest that the animals with gliosis demonstrated paralytic disturbances near the end of their lives. Because of these findings we are now proceeding to reinnoculate the suspicious specimens in a new set of animals and to attempt re-passage from the brains of the animals who died and whose brains revealed gliosis or who exhibited paralysis terminally. It should be noted that all histologic examinations were done blindly on these specimens and the code broken only after the designation of gliosis was determined.

Significance: Schizophrenia is one of the most prevalent and most disabling neuropsychiatric problems in the United States, and indeed in the world. Schizophrenia affects approximately one percent of the U.S. population. It is largely young people who are most affected and 20-25 percent of those effected are severely and permanently handicapped despite enlightened use of the most modern therapeutic measures. New approaches to both prevention and treatment are urgently required. The work is difficult and immediate rewards are few. Because of considerable circumstantial evidence for infection as a significant cause of some schizophrenias, we are focusing our efforts on the search for an infectious agent. We are fully aware that this is "long-shot" research with no immediate promise of answers. The evidence, however, in favor of such an etiology and the rapid proliferation of powerful new methods for investigation of an alien protein or an infectious particle encourage us to continue these efforts. Of special importance in making this effort is the unique opportunity offered by having in the NIMH and NINCDS a remarkable union of research material (brain bank) and voluntary research schizophrenic patients at Saint Elizabeths (source for CSF and serum) plus the outstanding virology laboratory and collaborators at NINCDS Intramural Program.

Proposed Course of Project: Our immediate plans for this project are as follows:

Schizophrenia-viral studies:
a) continue the genome collaboration studies with Dr. Haase - he has the frozen brain material for genome studies in hand.

b) Tissue cultures. This is now in progress in collaboration with Drs. Ziegler, Asher and Schwartz, as noted. We feel it is a real accomplishment to now have the cell lines growing and in progress, ready for co-cultivation should that opportunity arise. In the meantime, we continue to study the frozen material placed on the cultures. As we are blind to diagnosis it is too soon to speak of results. We have examined the effects (one case) and frozen (six cases) of brain specimens on neurons. Our future plans include the study of peptides, binding sites, and cDNA for specific peptide precursors on well-characterized cell lines presently being grown in the laboratory.

c) Passage of brain material from original specimens to animals. Drs. Kaufmann and Asher are carrying this work forward to attempt serial passage from brains in animals identified as neurologically afflicted on the basis of examination of course and post mortem tissue. The role of this investigator is to examine the pathologic material.

Publications:

Stevens, J.R., Langloss, J., Albrecht, P. and Yolken, R.: A search for cytomegalovirus and herpes viral antigen in brains of patients with schizophrenia. Arch. Gen. Psychiatry, 41:795-801, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 MH 02276-01 NPB

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Localization of Met^5-Enkephalin-Arg^6-Phe^7-Like Immunoreactivity

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

Yen-Nung Wang, M.D., Visiting Associate, NPB, IRP, NIMH

Dr. Allen Church, Staff Fellow, NPB, IRP, NIMH; Dr. Richard Jed Wyatt, Chief, Neuropsychiatry Branch, IRP, NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH
Neuropsychiatry Branch

SECTION
Section on Aging

INSTITUTE AND LOCATION
NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.50	.50	

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 Met^5-enkephalin-Arg^6-Phe^7 (Met-Enk-Arg-Phe)-like immunoreactivity in the rat gastrointestinal tract was studied by immunohistochemical techniques. Antiserum against a Met-Enk-Arg-Phe-throglobulin conjugate was raised in rabbits and was found to be specific for synthetic Met-Enk-Arg-Phe. Met-Enk-Arg-Phe-like immunoreactivity was found in neuronal structures in all parts of the rat gastrointestinal tract. Immunostained somata were primarily located in myenteric plexus; immunostained processes were mostly present in myenteric plexus and circular muscle layer. This distribution pattern is similar to that of the three other opioid polypeptides, Met^5-enkephalin, Leu^5-enkephalin and Met^5-enkephalin-Arg^6-Gly^7-Leu^8.

PHS 6040 (Rev. 1/84)

GPO 904-917

Project Description:

Objectives: The opioid heptapeptide, Met^5-enkephalin-Arg^6-Phe^7 (Met-Enk-Arg-Phe), was originally isolated from bovine adrenal medulla. The Met-Enk-Arg-Phe sequence has been found to be present in proenkephalin-A together with Met^5-enkephalin (Met-Enk), Leu^5-enkephalin (Leu-Enk) and Met^5-enkephalin-Arg^6-Gly^7-Leu^8-enkephalin (Met-Enk-Arg-Gly-Leu). Using radioimmunoassay (RIA) techniques Met-Enk-Arg-Phe has been quantified in brain, gastrointestinal tract, lung and other peripheral tissues of several species. Through the use of immunohistochemical techniques, Met-Enk-Arg-Phe has been found in the brain and recently in the gastrointestinal tract of the rat, mouse and guinea pig. The aim of the present study was to characterize the immunohistochemical localization of Met-Enk-Arg-Phe in the gastrointestinal tract and to compare it with some related opioid polypeptides.

Methods Employed: Adult Sprague-Dawley rats (150-200 g) of both genders were used. Under deep chloral hydrate anesthesia, rats were prefused with saline followed by 4% (w/v) paratormaldehyde with 0.1 M DL-lysine-HCl and 0.01 M sodium periodate in 0.1 M phosphate buffer, pH 7.4. Samples from esophagus, stomach, duodenum, jejunum, ileum and colon were removed, post-fixed for 2 h at 4°C and then stored at 4°C in 30% (w/v) sucrose in 0.1 M phosphate buffer. Cryostat sections were cut at 10 μm and placed onto gelatin-coated slides.

Tissue sections were processed for immunohistochemical staining. The sections were examined with a Zeiss Photomicroscope III equipped for incident light fluorescence examination.

Specificity of Met-Enk-Arg-Phe immunoreactive staining wsa tested by incubating section in the primary antiserum diluted 1:200 to 1:500 in phosphate buffer containing 0.3% (v/v) Triton X-100 and preabsorbed with one of following peptides (10 μm) for 48 h at 4°C: Met^5-Enk, Leu-Enk, Met-Enk-Arg-Phe, Met-Enk-Arg-Gly-Leu and Phe-Met-Arg-Phe-Ne_2 (FMRF-NH_2).

Findings: Met-Enk-Arg-Phe-like immunoreactivity was found in neuronal structures in all parts of the rat gastrointestinal tract. The number of immunoreactive nerve cell bodies and fibers was greatest in the colon, while in the gastric corpus, antrum, pyloric region, duodenum, jejunem and ileum, there were a moderate or few nerve cell bodies and fibers. In the esophagus we did not find any immunoreactive nerve cell bodies and only occassionally a fluorescent element that resembled a fiber.

Significance to Mental Health Research: Like Met-Enk, Leu-Enk and Met-Enk-Arg-Gly-Leu, Met-Enk-Arg-Phe is apparently another endogenous opioid peptide, with a possible neurotransmitter or neuromodulator function. It may prove important to the understanding of brain biochemistry in mental disorders to know what its physiological actions are in the gastrointestinal tract and how this heptapeptide may interact with other opioid peptides.

Proposed Course of Project: We plan to continue investigating the mechanisms of this opioide peptide throughout the next reporting year.

Publications:

Wang, Y-N., Church, A.C. and Wyatt, R.J.: Localization of Met^5-Enkephalin-Arg^6-Phe^7-like immunoreactivity in the rat gastrointestinal tract. Neurosci. Lett. 51:319-324, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 02277-01 NPB

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Regional Cerebral Blood Flow in Neuropsychiatric Patients and in Normal Subjects

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Daniel Weinberger, M.D., Chief, Section on Clinical Neuropsychiatry, NPB, NIMH; Karen Faith Berman, M.D., Staff Psychiatrist, NPB, IRP, NIMH

Dr. John M. Morihisa, Staff Psychiatrist, NPB, IRP, NIMH; Dr. Ronald F. Zec, Psychologist, NPB, IRP, NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH
Neuropsychiatry Branch

SECTION
Section on Clinical Neuropsychiatry

INSTITUTE AND LOCATION
NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.50	1.75	1.75

CHECK APPROPRIATE BOX(ES)

- [X] (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.)*

Using the Xenon133 inhalation technique, the regional cerebral blood flow (rCBF) lab within the Section on Clinical Neuropsychiatry, carries out investigations of rCBF (as an indicator of regional cortical metabolism) in a variety of neuropsychiatric patients and in normal subjects. Patient populations, including those with chronic schizophrenia, affective disorder, obsessive-compulsive disorder, Huntington's disease, Parkinson's disease, Alzheimer's disease, and dyslexia are studied before and during various exploratory and therapeutic interventions. Normal control subjects matched for each patient study are investigated concurrently. cortical metabolic concomitants of states of normal cognition and consciousness are also being explored. The Xenon133 method allows for multiple determinations of rCBF in a single individual who can, thus, serve as his or her own control while being studied serially under various cognitive and/or medication conditions. This allows paradigms to be designed to specifically test hypotheses about regional cortical function in disease states and normal higher cognitive function and to specifically monitor experimental and therapeutic inteven-tions in neuropsychiatric disorders. Careful and creative application of this versative tool has produced important results. Experiments tailored to explore dorsolateral prefrontal cortex (DLPFC), an area of special interest in schizophrenia, have shown this area to be de-activated in patients with schizophrenia under conditions of cognitively specific, regionally selective demand of this area - conditions under which normals increase metabolism to DLPFC. In contrast Huntington's disease patients who are as cognitively impaired as schizophrenics, do not show DLPFC rCBF abnormality, but rather rCBF patterns similar to normal subjects. This is important evidence for the existence of subcortical dementia, which, until now, has been questioned by some.

GPO 904-917

Project Description:

Objectives: Until recently, investigations of the human brain have been restricted to animal model facsimilies, subjective clinical observations of human subjects, and measurements of peripheral markers of CNS activity in the blood, urine, or CSF. However, new techniques for directly determining brain function in living human patients are now yielding important data about regional neurophysiology in normal function and in disease states. One such technique, Xenon133 inhalation rCBF, has proved particularly well suited to the study of neuropsychiatric disease. With this method rCBF, which has been shown to be tightly linked to local metabolic activity, can be measured in various patient populations in a relatively expedient, convenient, reliable, and inexpensive manner, and regional physiological correlates of normal brain function and neuropsychiatric disease can be determined. This is an essential prerequisite to the understanding and treatment of these disorders and has important potential implications for monitoring therapeutic interventions.

Using this technique the NIMH rCBF lab is endeavoring to delineate the regional neurophysiological concomitants of 1) normal brain function and aging, 2) neuropsychiatric illnesses including schizophrenia, affective disorder, multiple personality disorder, obsessive-compulsive disorder, Parkinson's disease, Huntington's disease, Alzheimer's disease, and dyslexia, and 3) even the sequelae of such brain insults as encephalitis and leucotomy. In addition to investigations of cortical function in these conditions, we are also undertaking the direct study of effects of pharmacotherapeutic interventions on cortical physiology and their relation to clinical response. These latter studies include evaluations of effects of standard medications, such as neuroleptics and anticholinergics in schizophrenia and levodopa/carbidopa in Parkinson's disease, as well as novel experimental treatments such as calcium channel blockers and arginine-vasopressin in schizophrenia and the muscarinic agonist, RS-86, in Alzheimer's disease.

To maximize the heuristic contribution of the regional neurophysiological data obtained, the rCBF team works with other investigators to build a multimodal data base consisting not only of rCBF data, but also correlative neurostructural data (via CT scanning), clinical and demographic information, other complementary measures of neurophysiology (PET and BEAM), and cognitive batteries.

Methods Employed: Regional cortical metabolism is determined using radioactive tracer kinetics principles. Following a one-minute inhalation of small concentrations (5-7 mCi/liter) of the physiologically and chemically inert and freely-diffusable radioisotope gas, Xenon133, rates of arrival and elimination of radioactivity in 32 cortical areas are monitored via an array of extracranial sodium iodide scintillation detectors for an additional 14 minutes. Subjects are typically studied under three different cognitive conditions during a single morning or afternoon session. The first of these is a "resting state" study primarily for purposes of acclimatization. Then rCBF is measured during two cognitive activation conditions that are presented in counterbalanced sequence. Usually these are paired tasks, one of which is tailored to activate the cortical region of interest to the disorder, therapeutic intervention, or normal function being investigated. The other task serves as a control for those aspects of the procedure that are non-specific and extraneous to the study. Various parameters of autonomic arousal including galvanic skin response, pulse, respiratory rate, and carbon dioxide level are monitored during each procedure.

Paradigms are designed to address specific research questions. Examples of past and ongoing paradigms (each of which and its specially designed control procedure are performed while rCBF is being measured) include: (1) to study dorsolateral prefrontal cortical function,

an automated version of the Wisconsin Card Sort (WCS) designed in this laboratory (on-going in schizophrenia, affective disorder, Huntington's disease, Parkinson's disease, and post-leukotomy), (2) to assess cortical concomitants of attention and mental effort, two versions of a visual continuous performance task (CPT) that differ in difficulty and the amount of sustained effort and attention required (on-going in normal subjects and schizophrenia during various medication protocols), (3) to assess non-regionally specific complex reasoning and medication-effects, split-pair automated versions of Ravens progressive matrices that can be carried out on consecutive days without learning effects (on-going in schizophrenia, Alzheimer's disease and Parkinson's disease during medication and placebo), (4) to assess cortical laterality automated semantic classification and line orientation tasks (on-going in dyslexia and normal subjects), (5) to study the cortical effects of emotional state, simulated anxiety/depression paradigms (carried out on trained psychodramatists).

Findings: Schizophrenia: A number of observations implicate dorsolateral prefrontal cortex (DLPFC) in schizophrenia, including clinical symptoms similar to those of frontal lobe disease, decreased relative DLPFC regional cerebral blood flow (rCBF), and animal studies suggesting a role for DLPFC in cognitive processes that are commonly impaired in chronic patients.

rCBF was measured with Xenon133 inhalation during various cognitive tasks. First, to assess DLPFC function 20 patients medication-free (DF) for at least four weeks, 24 on medication, and 25 normal subjects completed a three-test series: rCBF was determined initially during the resting state then while subjects performed, in counterbalanced sequence, an automated version of the Wisconsin Card Sort (WCS) to selectively test DLPFC, and a simple numbers matching task (NM) to control for non-DLPFC-related aspects of the procedure. Next, to assess the roles of attention and task specificity in DLPFC function, rCBF was measured in 17 DF patients and 18 controls during two versions of a visual continuous performance task (CPT), an attentional task not specific for DLPFC. Finally, to further determine DLPFC function during complex, but non-DLPFC-linked, reasoning, rCBF studies were done while subjects solved Raven's Progressive Matrices (RM). Brain structure was assessed with CT, in 18 DF and 22 medicated patients who completed the WCS/NM paradigm.

DLPFC rCBF was selectively decreased in DF and medicated patients specifically during WCS. No DLPFC difference between patients and controls was noted during NM, CPT's, or RM. Degree of DLPFC activation was correlated with patients' performance on the WCS but not with autonomic arousal. DLPFC rCBF correlated with several parameters of structural pathology in CT. These data suggest a pathophysiological mechanism for the cognitive impairment in schizophrenia.

Huntington's disease: The dementia of Huntington's disease (HD), though clinically familiar, has been difficult to characterize on a neuropathological basis. Despite the long-held assumption of the importance of cortical degeneration, evidence of a link between dementia and cortical atrophy is weak. PET studies of glucose metabolism in patients with HD suggest that, in contrast to striatum, cortex is normal in the resting state and during simple motor movements. The present study is an investigation of regional cortical blood flow (rCBF) in patients with HD during performance of a cognitive task on which they characteristically do poorly.

Ten patients (mean $\pm$ SD age 41 $\pm$ 12) and 15 normal volunteers underwent three Xenon133 inhalation rCBF procedures: first at rest and then, in counterbalanced sequence, while performing an automated version of the Wisconsin Card Sort Test (WCS), which selectively

tests dorsolateral prefrontal cortical function (DLPFC), and while performing a simple numbers matching (NM) task, which served as a control. At rest and during NM, grey matter CBF to prefrontal (DLPFC) and precentral regions both in absolute levels and as a ratio of non-frontal flow did not differ significantly between groups. Despite many more perseverative and conceptual errors on the WCS, the HD patients' prefrontal and precentral CBF was not different than normals during the test. Likewise, regional CBF % changes (WCS-NM)/NM did not differ significantly between groups. DLPFC relative flow correlated directly with WCS performance in both groups. In contrast, stage of illness (Shoulson and Fahn), while correlating with WCS performance, did not correlate with rCBF. These results, by suggesting normal cortical metabolism during abnormal cognitive function, provide additional evidence for the role of subcortical pathology in the dementia of HD. Because of caudate pathology, prefrontal cortex is presumably de-efferented.

Significance to Mental Health Research: The regional pathophysiology underlying neuropsychiatric disorders, and even that accompanying normal higher cognitive function or emotion, is poorly understood. The rCBF lab within the Section on Clinical Neuropsychiatry has a unique opportunity to directly study CNS phenomena in a variety of clinical populations, to elucidate normal neurophysiology and to directly monitor the CNS effects of therapeutic interventions. Furthermore, by elaborating a multimodal correlative data base of cognitive, structural, and clinical measures we are better able to interpret our rCBF data in a broader perspective. The opportunity to search for commonalities and differences in a wide variety of patient populations and clinical states maximizes the potential of this work to delineate the regional underpinnings of CNS pathophysiology as well as normal function. Thus, our ultimate goal is to better understand the until-now elucive workings of the living human brain in health and in disease states, and to sucessfully intervene in the latter.

Proposed Course of Project: The first rCBF procedure was carried out in March, 1983, and since that time over 950 individual procedures have been successfully completed in the rCBF lab. Data management techniques for the resultant extensive informational base and statistical methods to address the complex interrelationships of regional brain function have been and continue to be developed. Important research questions have been formulated, and in some cases the answers have lead to new questions. In addition to the continuation of the projects described above, new protocols to evaluate the effect of clonidine in Alzheimer's disease are anticipated.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02278-01 NPB

PERIOD COVERED

October 1, 1984 through September 30, 1985

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

Structural Brain Imaging in Schizophrenic Patients and Normal Subjects

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

Daniel R. Weinberger, M.D., Chief, Section on Clinical Neuropsychiatry, NPB, IRP, NIMH

Dr. Richard C. Shelton, Clinical Research Associate, NPB, IRP, NIMH; Dr. Jack A. Grebb, Clinical Research Associate, NPB, IRP, NIMH; Dr. Dilip V. Jeste, Medical Officer, NPB, IRP, NIMH; Dr. Ronald F. Zec, Psychologist, NPB, IRP, NIMH; Dr. Richard Jed Wyatt, Chief, Neuropsychiatry Branch, IRP, NIMH; Dr. John M. Morihisa, Staff Psychiatrist, NPB, IRP, NIMH; Dr. Allen Doran, Clinical Research Associate, Clinical Neuroscience Branch, NIMH; Dr. David Pickar, Chief, Section on Clinical Studies, Clinical Neuroscience Branch, NIMH

COOPERATING UNITS *(if any)*

Clinical Neuroscience Branch, NIMH; George Washington University;

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Section on Clinical Neuropsychiatry

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.75	1.25	1.50

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 on structural brain imaging investigates structural pathology of the brains of schizophrenic patients housed in the William A. White research units using x-ray computerized tomography (CT). Patients are compared to matched normal controls. The most recent study, a culmination of four years of data collection, compared 73 schizophrenic patients to 30 normal volunteer controls. This project is a replication and extension of the previous work done in this area in the branch. Using standardized techniques four brain areas were examined: lateral ventricles, third ventricles, cortical (parieto-occipital) areas, and prefrontal cortex. In this sample, the lateral and third ventricles continued to be significantly larger in patients than controls. A potentially exciting new finding was that though there were essentially no differences between patients and controls in cortical atrophy in the parieto-occipital distribution, the schizophrenic patients showed substantially greater atrophy in the prefrontal distribution, localizing the cortical changes to this area. Further, in a subgroup of 18 drug-free and 22 medicated patients, the CT abnormalities were correlated with regional cerebral blood flow (rCBF) using the radioactive ^{133}Xenon inhalation technique. Relationships were found between the neurophysiological measurements of CT scanning, especially in the prefrontal cortex and ventricular areas.

PHS 6040 (Rev. 1/84) GPO 904-917

Collaborators:

Dr. Carl Feinstein, Ass. Professor of Psychiatry, George Washington University
Dr. Steven G. Potkin, Staff Psychiatrist, NPB, IRP, NIMH

Project Description:

Objectives: Before the advent of CT scanning, observations of the human brain were largely indirect: chemical markers of brain metabolic activity in the cerebrospinal fluid, blood, or urine; responses to centrally-acting medications; animal models of human brain function; post-mortem brain studies; and crude methods of visualizing brain structures such as pneumoencephalography. CT scanning proved a major advance in providing detailed pictures of cross-sections of brain in living subjects with minimal risk. These accurate and reliable methods could then be applied to the study of brain pathology in such diseases as schizophrenia.

In the past this laboratory has used CT observations to study a number of parameters of brain structural abnormalities in schizophrenic patients. Reversed cerebral asymmetries and cerebellar atrophy have been described in patients, but the first and most venerable findings were those implicating atrophy of the cerebral cortex. In particular, previous studies have indicated enlargement of lateral and third ventricles and atrophy of the cortical surface, findings apparently unrelated to age, neuroleptic exposure or duration of illness but apparently related to severity of illness as described by poor premorbid adjustment, diminished response to neuroleptics, poorer outcome, cognitive impairment, and other positive symptoms. Additionally, these changes are apparently present at the onset of illness.

Over the last year the laboratory has tried to meet several objectives: (i) to replicate the previous work showing cerebral atrophy in a sample of schizophrenic patients more representative of the broad distribution of affected persons. (ii) to extend the work by attempting to localize the site of cortical atrophy and (iii) relate the changes on CT scan to physiological measures by rCBF, especially so-called "hypofrontality" (i.e., relatively diminished prefrontal blood flows under conditions of cognitive stimulation of this area).

To achieve these results, CT and rCBF data were collected along with clinical information and neuropsychological testing.

Methods Employed: Patients selected for study were housed in the clinical research units, Saint Elizabeths Hospital, William A. White Building, and were rigorously diagnosed as having schizophrenia by DSM-III criteria. Normal volunteer controls were obtained via several investigation at the NIH Clinical Center, Bethesda, Maryland. All subjects underwent standard CT scanning with the same GE 8800 scanner at the Clinical Center. Twelve to 13 images or slices were produced at 15^{o} to the cartho-meatal line. Measurements were made from these images on photographic film.

Lateral ventricle size was measured using a fixed-arm planimeter, an engineering device used to measure the area of irregular two-dimensional structures. The ventricular area is divided by the area of the whole brain, multiplying by 100, giving a percentage size or ventricular-brain ratio (VBR). Third ventricular size is measured by laying a mm ruler across the greatest diameter, then multiplying by a so-called "minification factor" of 2.7 (the relationship between the photographic image and the true size of the subject's brain). Generalized (parieto-occipital) atrophy was evaluated on an appropriate slice with a 0 (mild) to 3 (severe) scale with half-steps beteen (e.g., 0.5, 1.5, 2.5) by referring to standard examples. Prefrontal atrophy was similarly evaluated on a scale derived from CT cuts at an appropriate position. The patient data derived were compared to the same measurements from volunteer controls. All measurements were performed blind to patients vs controls.

A subsample of patients were selected to compare CT changes and abnormalities on rCBF described in detail under another heading.

Major Past Findings: In a relatively severely-impaired sample of patients, there was evidence of abnormal enlargement of lateral and third ventricles and cortical atrophy (measured with a different scale). These abnormalities did not relate to clinical parameters such as age, duration of illness or hospitalization or neuroleptic treatment, but were correlated with poor premorbid adjustment, cognitive impairment, poor response to neuroleptics and poor outcome. Other abnormalities discovered included an increased incidence of cerebellar atrophy and reversed cerebral asymmetries in the patients. Additionally, evidence of prefrontal atrophy was correlated with increased delta- or slow-wave activity in the prefrontal area as assessed by brain electrical activity mapping (BEAM), a computerized evaluation of the electroencephalogram.

New Findings: Comparing the 73 schizophrenic patients to 30 normal volunteer controls, enlargement of lateral and third ventricles was again demonostrated; as predicted this was to a lesser degree than described before in this somewhat less-severely impaired patient sample, though still reaching statistical significance. There were no differences between patients and controls on the generalized (parieto-occpital) scale, but significant differences in the prefrontal distribution indicating a localization of atrophy in this area. This is consistent wtih some findings from rCBF, BEAM, Positron Emission Tomographic Scanning and Neuropsychological deficits found in schizophrenic patients. This is also significant in light of the similarities of symptoms found in persons with known injuries of the dorsolateral prefrontal cortex and the so-called "core" or "defect" symptoms of schizophrenia including flattened affect, social impairment, apathy, withdrawal, etc.

Abnormalities on rCBF, in particular the so-called hypofrontality seen in schizophrenic patients under conditions of specific neuropsychological stimulation (the Wisconsin Card Sorting test, an activator of the dorsolateral prefrontal cortex) were compared to structural abnormalities on CT scan in 18 drug-free and 22 medicated patients. Decreased relative prefrontal blood flow was found to correlate with prefrontal atrophy and strongly with lateral ventricular enlargement in all patients. This seems to indicate relationships between physiological dysfunction in the prefrontal cortex and structural abnormalities in both prefrontal and subcortical areas. This is consistent with a developing knowledge base from basic research indicating strong interrelationships between prefrontal and subcortical (periventricular) structures that may be abnormal in schizophrenia.

Significance to Mental Health Research: Understanding the basic structural and physiological dysfunction in the brains of schizophrenic patients is vital to the progress of research in the illness. Such underpinnings will allow the development of specific neurorehabilitative paradigms and potentially an understanding of the etiologies of the illness. By comparing structural and functional measurements, abnormalities can be better localized int he brain, and can be related to specific clinical parameters. The primary goal, then, is to clarify the site of the "critical lesions" in the brains of schizophrenic patients, i.e., those areas primarily effected in the illness, accounting for the core symptoms.

Proposed Course of Project: With the extensive CT data base collected, correlations will be made with various clinical and psychological parameters, e.g., cognitive impairment, "negative" and "postive" symptoms and neuroleptic responsiveness. Plans are also being formulated to utilize an exciting new imaging technique, nuclear magnetic resonance (NMR). This technique will allow structural imaging in exquisite detail, revealing more

specifically areas such as individual periventriclar nuclei, depth of cortical and periventricular gray matter, and giving a much more specific "look" at brain structural abnormalities.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02279-01 NPB

PERIOD COVERED

October 1, 1984 through September 30, 1985

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

Comparison of Neuroleptic Induced Supersensitivity in Mice

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

Alex Wisniewski, M.D., Medical Staff Fellow, NPB, IRP, NIMH

Dr. Dilip V. Jeste, Medical Officer, NPB, IRP, NIMH; Dr. Richard Jed Wyatt, Chief, Neuropsychiatry Branch, IRP, NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Section on Aging

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.20	.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.)*

Two inbred strains of mice were studied for possible differences in neuroleptic induced supersensitivity. Six mice from each strain were treated with Prolixin decanoate for six weeks and baseline and post-apomorphine activity counts were measured to establish degree of supersensitivity. Statistically significant differences were obtained between the two strains.

PHS 6040 (Rev. 1/84)

GPO 904-917

Project Description:

Objectives: The primary objective of this project is to examine supersensitivity induced by neuroleptics in mice.

Methods Employed: Behavioral counts of locomotion will be obtained on Jackson Laboratory mice, both before and after administration of neuroleptics.

Major Past Findings: Differences between the two strains of mice studied (BALB/cBy and C57BL/6By) in terms of brain D2 receptors have been estimated at 30%. Supersensitivity and measure of its degree by using dopaminergic agonists such as apomorphine has been demonstrated in animal research by numerous authors and neuroleptic (i.e., fluphenazine) induced supersensitivity has been used as a model for tardive dyskinesia for over a decade.

New Findings: Supersensitivity induction was shown to differ in strains of mice known to have D2 receptor density variation.

Significance to Mental Health Research: Utilizing animals known to differ in dopaminergic recpetors may illustrate how supersensitivity develops following neuroleptic administration. Clinicians observe different responses to neuroleptic between individuals and this may be determined by the initial receptor density. If supersensitivity develops different in the two groups it could explain why some individuals run the risks of developing side effects.

Proposed Course of Project: Mice will be exposed to neuroleptic for six weeks and examined by apomorphine challenge. Further testing will be obtained using adrenergic stimulants such as clonidine. The project may expand to use other receptor challenges to examine supersensitivity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 MH 02280-01 NPB

PERIOD COVERED

October 1, 1984 through September 30, 1985

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

Brain Tissue Transplantation in Primates

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

Richard J. Wyatt, M.D., Chief, Neuropsychiatry Branch, IRP, NIMH

Dr. William J. Freed, Chief, Section on Preclinical Neurosciences, NPB, IRP, NIMH; Dr. J.M. Morihisa, Staff Psychiatrist, NPB, IRP, NIMH; Dr. Richard Nakamura, LPP, IRP, NIMH; Dr. Donald Price, Johns Hopkins Hospital; Dr. Cheryl Kitt, Johns Hopkins Hospital.

COOPERATING UNITS *(if any)*

LPP, NIMH
Johns Hopkins Hospital

LAB/BRANCH

Neuropsychiatry Branch

SECTION

INSTITUTE AND LOCATION

NIMH, NIH, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.50	.50	

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 advance the work already performed in our laboratory with rats, fetal substantia nigra or adrenal medulla was grafted to the denervated caudate of the rhesus monkey in our continuing research on brain tissue transplantation. Although only moderately suggessful thus far, the experiments have demonstrated for the first time that peripheral tissue autografts can survive transplantation into nonhuman primate central nervous system.

PHS 6040 (Rev. 1/84)

GPO 904-917

Project Description:

Objectives: The objective of this program is to take knowledge gained from brain tissue grafts in the rodent into primates and eventually into humans.

Methods employed: These studies involve surgical, histological and histochemical procedures in primates.

Major finding: Considerable success has been achieved in grafting embryonic substantia nigra in young adult adrenal medulla in rats to decrease rotational behavior produced by unilateral lesions of the substantia nigra.

Prior to introducing brain grafts in humans, it is important to establish procedures in animals intermediate between the rat and man. For example, it is crucial to know if grafts survive and if so, how well in nonhuman primates. Our first series of rhesus macaque (Macaca mulatta) animals using both adrenal medulla and rhesus embryonic substantia nigra was, for the most part, unsuccessful. With one exceptions, grafts were not found.

A second series has been slightly more positive; at least some tissue survived transplantation. Eight mature adult male rhesus macaque animals received a unilateral neurotoxic lesion of the region of the substantia nigra (including A10). At least two months later, each animal received a unilateral implant of either fetal rhesus substantia nigra tissue or tissue from its own adrenal medulla, and at least two months after implanttion each animal was killed for catecholamine fluorescence histochemistry. The first two animals (A1 and A2) received fetal substantia nigra. The remaining animals (A3 through A7) received host adrenal medulla tissue.

For the embryonic substantia nigra implants, a rhesus monkey embryo (59 and 71 day) was surgically removed ex utero and placed in lactated Ringer's solution. The brain was dissected to obtain substantia nigra tissue in a manner analogous to that for embryonic rat brain. The region of the midbrain that inludes substantia nigra tissue was divided into approximately 0.25 mm^3 pieces. This tissue was drawn into a 22-guage needle with an average of six pieces of tissue per injection in a volume of approximately 10 to 20 ul of Ringer's solution.

After removing bone and dura, the injection needle was lowered into the head of the caudate nucleus with stereotaxic coordinates corrected by X-ray determination of skeletal landmarks. The needle was lowered until it was within the caudate. The tissue was then injected and after 3 min the needle was withdrawn.

For the six remaining animals (A3 through A8), the left adrenal was taken through a posterolateral retroperitoneal approach. A single longitudinal incision was made through the adrenal capsule and cortex under a dissecting microscope. The adrenal cortex was peeled off and cortical fragments trimmed away. The adrenal medulla was divided into pieces of 0.25 mm^3. The tissue in Ringer's solution was drawn into a 22-gauge needle with between 5 and 10 pieces of tissue in a volume of approximately 10 to 20 ul per injection.

In animal A3, stereotaxic placement of the adrenal graft was used as described above for the two animals implanted with fetal substantia nigra. A

total of five injections were made into the caudate of animal A3. In the five remaining animals (A4 to A8), to provide more secure anatomic placement of grafts, the caudate was directly observed. With the aid of a surgical microscope, a window was cut through the body of the corpus callosum exposing the left lateral vehtricle and caudate. A 22-guage needle was inserted into the body of the caudate to inject the tissue.

Surviving graft tissue could be identified by the presence of specific catecholamine histofluorescence in the cell bodies of the implants. Neither animal implanted with fetal substantia nigra had any evidence of surviving catecholamine-containing graft tissue. In contrast, the six animals implanted with host adrenal medulla had at least some surviving tissue in the parenchyma of the denervated caudate nucleus. The graft tissue itself appeared relatively healthy, although accumulation of macrophages was noted adjacent to or surrounding some of the graft sites. The only damage to host caudate associated with the implantation procedure was scar formation along the needle track.

Most graft sites were deep within the body of the caudate nucleus along the implant tract. Additionally, there were two graft sites that were on the edge of the caudate nucleus. At least some parts of most grafts appeared fused with the brain parenchyma, but there was no evidence of caudate reinnervation. All graft sites demonstrated some diffusion of the catecholamines. Most fluorescent cells retained the typical rounded appearance of adrenal chromaffin cells. A minority of cells developed polygonal shapes, and a few cells appeared to develop nerve-like fiber processes, though these remained within the graft itself. A third series of animals has been more successful but results are preliminary.

In addition to grafting tissue directly into the striatum of monkeys, tissue has been grafted into the frontal cortex. The advantage of grafting into the frontal cortex is that the surgery is considerably simpler than grafts into the striatum, and allows for developing surgical procedures which do not require lesioning animals and use of complex stereotaxic placement of grafts. In addition, in some cases animals do not need to be sacrificed to determine results. In at least two animals in this series over 10,000 adrenal chromaffin cells have survived.

Significance to Mental Health Research: These studies may lead to the development of tissue transplantation as a therapeutic procedure for degenerative diseases and destructive lesions of the brain in the clinic. Also, they may lead to increased knowledge about development and regeneration in the brain in general. Since there is considerable evidence that some schizophrenic patients have altered brain structure, perhaps through degeneration, and degeneration is clearly involved in diseases such as Alzheimer's disease, learning more about brain plasticity is of primary importance to understanding these illnesses.

Proposed Course of Project: Brain grafting should be seen as both a potential treatment for disorders such as Parkinson's disease as well as a potential for understanding plasticity in general. The course of this project should continue until such time as there is sufficient justification for bringing these techniques into the clinic. At that time further refinements and developments will probably be needed in order to maximize potential benefits to

patients. Because work with primates is inherently slow, progress will also be slow, but nevertheless there does appear to be incremental enhancements of our ability to graft tissue in primates over the last few years. We would expect this progress to continue over the next several years.

Publications:

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02281-01 NPB

PERIOD COVERED

October 1, 1984 through September 30, 1985

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

Neural Tissue Microchip Interface

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

Richard Jed Wyatt, M.D., Chief, Neuropsychiatry Branch, IRP, NIMH

A. Paul Oliver, Physiologist, NPB, IRP, NIMH; and Dr. Marty Peckerar, Naval Research Laboratory, Washington, D.C.

COOPERATING UNITS *(if any)*

Naval Research Laboratory

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.25	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.)*

Communication between elements of the central or peripheral nervous system and electronic systems, principally computers, is needed to develop advanced prosthetics. Crude prosthetics such as the artifical ear have already been used clinically. This program tests the interaction of brain tissue with solid state interface devices to make multi-channel contacts between brain and computer a reality. Work at this stage demonstrates the feasibility of the concept.

PHS 6040 (Rev. 1/84)

GPO 904-917

Project Description:

Objectives: Brain tissue that is destroyed cannot be regrown. It is possible to restore some function with prosthetics. Computers are fast enough and complex enough currently to replace some neuronal function. In the course of this project we will explore the technical and conceptual problems of using solid state devices to enhance or replace function. A very important benefit of this project will be its usefulness in basic research on brain function. This program, undertaken with the cooperation of the Naval Research Laboratory, is intended to develop, fabricate, and test interface devices for neuroelectronic communication.

Methods Employed: The first device we have developed is a chip in which 30 microelectrodes are implanted. The chip, fabricated at the Naval Research Laboratory forms the bottom of a tissue culture chamber. Nerve cells from fetal rat brain are grown directly on the chip. The chip-chamber combination is mounted in a circuit board that provides part of the electronic interface for a computer. The electronic interface was designed and fabricated by NIMH Technical Development personnel.

Findings: Nerve tissue can be grown on the chip and individual nerve cells can be recorded via the chip interface. To date the gold silicon technology is stable in a long term biological environment.

Significance to Mental Health Research: The processing of information in the central nervous system is not well understood and is difficult to replace when the system is damaged. Most of the methods currently used to study CNS and to attempt functional replication are slow in gathering quantities of information, and in using it in real time. The development of multi-channel studies coupled with real time computer communication may contribute to a resolution of these problems.

Proposed Course: The current instrument in use is a practical benchtop device that can be used in any laboratory. In future the lessons learned from its use will be transfered to the design of a multi-channel probe for use in vivo.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02282-01 NPB

PERIOD COVERED

October 1, 1984 through September 30, 1985

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

Neurovirology and Neuroimmunology of Schizophrenia

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

Charles A. Kaufmann, M.D., Senior Staff Fellow, NPB, IRP, NIMH

Dr. Daniel R. Weinberger, Chief, Section on Clinical Neuropsychiatry, NPB, NIMH; Dr. Joel E. Kleinman, Chief, Section on Clinical Brain Studies, NPB, NIMH; Dr. Darrell G. Kirch, Senior Staff Fellow, NPB, NIMH; Dr. Lynn E. DeLisi, Medical Officer, Clinical Neurogenetics Branch, NIMH; Dr. Janice R. Stevens, Guest Researcher, NPB, NIMH; Dr. E. Fuller Torrey, Medical Officer, Saint Elizabeths Hospital; Dr. Nicholas M. Papadopoulous, Senior Staff Member, Clinical Pathology Department, NIH

COOPERATING UNITS *(if any)*

Clinical Neurogenetics Branch, NIMH, NINCDS, AFIP, Johns Hopkins University

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Clinical Neuropsychiatry Section

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.40	1.40	

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 on the neurovirology and neuroimmunology of schizophrenia is aimed at providing direct evidence for or against transmissible agents in the pathogenesis of schizophrenia, at examining immune dysfunction reflective of, or predisposing to, viral infection in schizophrenia, and at delimiting the effects of neuroleptic medications and prolonged hospitalization on the immune system. It is also amined at developing suitable models for studying the non-cytopathic effects of viruses on the central nervous system.

PHS 6040 (Rev. 1/84) GPO 904-917

Collaborators:

Dr. D. Carleton Gajdusek, Chief, Lab. CNS Studies, NINCDS
Dr. C. Joseph Gibbs, Jr., Deputy Chief, Lab CNS Studies, NINCDS
Dr. David M. Asher, Research Medical Officer, Lab CNS Studies, NINCDS
Lt. Colonel Joseph E. Parisi, Chief, Department of Neuropathology, AFIP
Dr. John Langloss, Chief, Department of Immunology, AFIP
Dr. Robert H. Yolken, Department of Pediatrics, Johns Hopkins University
Dr. William J. Smith, Department of Surgery, Johns Hopkins University
Dr. Susan E. Folstein, Department of Psychiatry, Johns Hopkins University

Objectives: Schizophrenia is a syndrome, the symptoms of which may result from a variety of insults (metabolic, toxic, traumatic, or infectious). Viruses are especially appealing as etiologic agents in schizophrenia: they have been implicated in the pathogenesis of several neuropsychiatric disorders, may be neurotropic (often for limbic structures thought to underlie the symptoms of schizophrenia), and allow for genetic contributions to disease expression (through susceptibility to infection or vertical transmission).

Phenomenological, epidemiological, neurochemical, neuropathological, and immunological data suggest a role for transmissible agents in schizophrenia. Phenomenology-psychotic symptoms have been associated with acute and chronic herpes (HSV) encephalitides and include bizarre delusions, auditory hallucinations, incoherence, along with blunted affect and catatonic stupor. These symptoms are reminiscent of both productive and deficit symptoms in schizophrenia. Moreover, schizophrenia has a unimodal age of onset, consistent with an infectious disease conferring life long immunity. Epidemiologically-schizophrenia is characterized by incomplete concordance between monozygotic twins, suggesting an interaction of genetic with environmental factors. Schizophrenia may be associated with a north/south prevalence gradient, possibly reflecting differences in virus range. Schizophrenia may also be associated with geographic and time clusters, compatible with epidemic outbreaks of the disorder. In addition, persons who develop schizophrenia are born disproportionately more often during the late winter or early spring months, consistent with prenatal exposure to a seasonal virus. Neurochemical-alterations in dopamine synthesis and turnover have been associated with acute and chronic herpes encephalitis in mice. These changes (for example, initial increases in turnover followed by decreases in synthesis) are similar to alterations in dopamine neurotransmission in schizophrenia. Neuropathology-CT scan evidence of increase lateral and third ventricle size and of enhanced periventricular radiodensity in schizophrenia suggests subcortical inflammation, is consonant with postmortem findings of periventricular fibrillary gliosis, and resembles the ventricular enlargement of congenital cytomegalovirus (CMV) infections and the periventricular inflammation of disseminated CMV infections in immunocompromised adults. Immunology-elevations in cerebrospinal fluid (CSF) antibodies (neutralizing and IgM) to CMV in schizophrenia suggest active or past infections with this virus or a related herpes virus. In addition, alterations in T cell number, morphology, or mitogen responsiveness have been described in schizophrenia and may either result from active infection of lymphoid tissue or result in disordered immune surveillance allowing persistent infection.

Such evidence, however, is indirect and does not distinguish between a causal and epiphenomenal role for viruses in schizophrenia. We have therefore undertaken a series of studies aimed at providing direct evidence for or against transmissible agents in the pathogenesis of schizophrenia. These studies have included attempts to isolate and transmit virus, and to detect viral antigens and genomes. Moreover, we have sought more a complete understanding of immunologic abnormalities in schizophrenia, examining the relationship between alterations in humoral immunity and neurochemical and neuropathological findings, and between alterations in cell mediated immunity and "non-specific" factors (such as neuroleptic treatment and hospitalization). Finally, we have begun to explore in vitro and in vivo models of virus-neuron interaction in an effort to define potential pathogenic mechanisms whereby viruses might effect CNS functioning in schizophrenia. These three areas of interest: viz. studies of transmissible agents in schizophrenia, studies of humoral and cell mediated immunity in schizophrenia, and models of virus neuron interaction will be discussed separately.

Studies of Transmissible Agents in Schizophrenia
(in collaboration with Lab CNS studies NINCDS and AFIP)

Methods Employed: Isolation-in an effort to replicate earlier findings of a "virus-like agent", resembling a picornavirus, in the CSF of schizophrenic patients, we examined the frozen CSF of 23 neuroleptic-free schizophrenic (DSM III) patients for its ability to induce cytopathic effects in MRC-5 tissue cell culture. Transmission-given the success of intracerebral inoculation in establishing the infectious etiologies of kuru and Creutzfeldt Jakob disease, we undertook similar attempts to transmit schizophrenia from the brains of patients to a limited number of laboratory animals, presuming that transmission might be recognized by the appearance of gross neurological changes or of neuropathologic changes which, while not pathognomonic, have been repeatedly described in patients with schizophrenia. Brain specimens were obtained within 24 hours of death from 10 patient (5 men, 5 women, mean age 43) who met diagnostic criteria (RDC) for schizophrenia or unspecified functional psychosis, probable schizophrenia. Patients had been chronically ill (mean duration 19 years) and had manifested both productive and deficit symptoms. Tissues for inoculation (frontal, temporal, parietal, occipital, cingulate cortex, septum, caudate, hippocampus, hypothalamus, and pons) were pooled and frozen. Fifty-nine animals, including six apes, 12 old world monkeys, 19 new world monkeys, and 21 guinea pigs were inoculated intracerebrally with freshly thawed tissue prepared as a 5%-20% suspension. Animals were observed regularly for signs of abnormal behavior and neurological disease. In a controlled neuropathologic study, nine guinea pigs, receiving inoculation from schizophrenic patients, were matched for age and cause of death with nine guinea pigs which had remained asymptomatic following inoculation with brain suspensions from patients with a variety of other (non-tranmissible) neurologic disorders. Brains were sectioned and stained with hematoxylin and eosin, Nissl's stain, Bodian's silver stain, phosphotungstic acid hematoxylin and by the avidin biotin immunocytochemical method with antibodies to glial fibrillary acid protein (GFAP). Morphometric analyses were performed on purkinje cells (cerebellar vermis) and pyramidal cells (hippocampus zones H1, H2, H3, H4-5) of schizophrenia inoculated and control guinea pigs. In addition, brains of four inoculated squirrel monkeys were compared with those of three control inoculated squirrel monkeys and two uninoculated squirrel monkeys. Detection of viral antigens and genomes-these complimentary studies have been undertaken by Dr. Janice R. Stevens in collaboration with Dr. Ashley Haase. Dr. Stevens has examined the paraffin embedded brains of 12 chronic schizophrenic patients (mean age 38, mean duration 11 years) 17 non-schizophrenic psychiatrically ill patients dying at the same hospital, and 10 non-psychiatric general medical hospital controls, with the highly sensitive peroxidase antiperixodase immunocytochemical technique with high titer anti CMV and anti HSV I and II IgG. Her collaborator, Dr. Haase has examined the frozen brains of 10 schizophrenic patients and 10 non-psychiatric controls matched for age, postmortem interval, and mode of death by *in situ* to hybridization with a ^{3}H-DNA probe to human CMV.

Major Past Findings: Isolation we were unable to replicate earlier studies which found cytopathic effects in tissue culture exposed to CSF from schizophrenic patients.

New Findings: Transmission-to date 19 guinea pigs and 14 primates have died. No consistent behavioral abnormalities have emerged, although one guinea pig and two squirrel monkeys developed progressive neurological symdromes characterized by tremors and gait disturbance at 20, 44, and 64 months after inoculation, respectively. Moreover, a second guinea pig (receiving the same inoculum as the symptomatic guinea pig and one of the symptomatic squirrel monkeys) remained asymptomatic until death 40 months after inoculation. Histopathological examination of rodent brains showed hippocampal and cerebellar gliotic changes

in five of nine experimental cases (including the symptomatic guinea pig). These changes were not specific, however, being found in three of nine control animals. On morphometric analyses, experimental and control animals did not differ in purkinje cell or pyramidal cell density size or nuclear size. Similarly brains of schizophrenia inoculated primates could not be distinguished from those of controls. These findings do support a role for transmissible agents, but must be weighed against methodologic limitations in animal susceptibility, disease communicability, and assay sensitivity. Diseases of known viral etiology, such as SSPE and progressive multifocal leukoencephalopathy (PML), have not been transmitted by these techniques. Their viral origin, suggested by electron microscopy, awaited confirmation by co-cultivation. Reisolation and blind passage attempts are underway, using material from acutely ill patients, and from symptomatic rodents and primates, respectively. In addition, electron microscopic examination of frozen postmortem material (including amygdala, dorsolateral pretrontal cortex, globus pallidus, hippocampus, nucleus accumbens, parahippocampal gyrus, septal nuclei, and substantia nigra) from four schizophrenic patients and three controls is being undertaken by Urmi Patel, Ph.D. to identify among other things, characteristic virions. Moreover, attempts to co-cultivate fresh specimens of parahippocampal gyrus obtained steriley at autopsy with various tissue cell lines are planned. A variety of different lines will be used initially to increase the likelihood of detecting different genuses of virus: specific lines will be chosen if electron microscopy indicates a specific type of virus. Detection viral antigens and genomes-in Dr. Stevens study, no section from basal forebrain, basal ganglia, or limbic structures from schizophrenic patients exhibited positive staining with IgG to CMV. Questionable staining of neurons and neuropil was noted in the lateral vestibular nucleus of one patient. In her collaborative study with the Haase, no section from substantia innnominata or septal region revealed hybridization with the DNA probe to human CMV. Once again these studies are limited-tissues from acute patients would be most likely to reveal either viral antigens or genomes. Unfortunately, only tissues from chronically ill patients have been available for study. In addition, only limited brain regions have been examined-affected areas may have been missed. Studies of more acute patients, examining additional regions for viral genomes are underway.

Studies of Humoral and Cell Mediated Immunity in Schizophrenia

Methods Employed: In an attempt to replicate earlier findings of CSF antibodies to CMV in patients with schizophrenia, and to relate any immunoglobulin abnormalities to structural and neurochemical changes, we examined the CSF of 35 (DSM III) schizophrenic patients and six neurologic controls for IgM against CMV antigen by enzyme linked immunosorbent assay. Serum and CSF of schizophrenic subjects were also analyzed by single radial immunodiffusion for total IgM and by HPLC with electrochemical detection for homovanillic acid. Patients also underwent (blind) assessments of CT scans. In a complementary study, we have considered if structural brain changes on CT scans may be static sequelae or the dynamic consequences of a continuing destructive process (like a viral infection). In collaboration with Dr. Anne Marie Duchemin and Dr. Tam Quach we have examined CSF of patients with and without evidence of CT abnormalities for the presence of "neuronotrophic" factors (factors which support the growth of 12 day chick embryo sympathetic ganglia neurons): similar factors are thought to appear within two days and disappear within two weeks of acute head trauma and, if present, would suggest an ongoing pathogenic process. In a third study designed to assess the integrity of the blood brain barrier (BBB), and the extent of intrathecal immunoglobulin synthesis, Dr. Darrell Kirch, Dr. Nicholas Papadopoulous, and myself examined the CSF and serum from 24 schizophrenic patients by rate nephelometry. CSF of patients in whom increased intrathecal IgG synthesis (elevated "IgG index") was found underwent additional analysis by high resolution protein electropheresis to identify oligoclonal protein

bands. A fourth study, undertaken in collaboration with Drs. Smith and Folstein at Johns Hopkins University has examined cell mediated immunity, specifically peripheral blood T lymphocyte subsets (determined by monoclonal antibodies and fluorescence microscopy), in 32 patients with schizophrenia, 15 normal volunteer controls, and 16 Huntington's disease controls (chosen because of history of hospitalization and neuroleptic treatment which paralleled that of schizophrenic subjects).

Major Past Findings: In our study of CSF antibodies to CMV, we did not detect a difference in CSF IgM between schizophrenics and neurological controls. Among schizophrenic subjects, however, IgM seem to be associated with CT scan evidence of ventricular enlargement, cortical (and cerebellar) atrophy or both ($p < 0.05$, Fisher exact test). Moreover IgM positive patients had lower CSF homovanillic acid than IgM negative subjects. While these findings may be related to non-specific polyclonal B cell activation known to accompany destructive lesions of the CNS, they also are consistent with virus mediated damage to brain structures and to dopaminergic pathways.

New Findings: In a pilot study of CSF neuronotrophic factors, we saw increased activity in two of three patients with enlarged ventricles and none of four patients with normal ventricles ($p=0.06$, Sign test). We are currently replicating the study with the CSF of 24 additional patients with or without large ventricles. In our third study of BBB integrity and intrathecal immunoglobulin synthesis, we found increased BBB permeability (compared with previously published age specific reference values) in seven of 24 (29%) of schizophrenic subjects. Eight of 24 (33%) of patients demonstrated elevated endogenous IgG production. One of the eight also demonstrated oligoclonal banding on high resolution protein electropheresis of CSF. This patient has recently agreed to return for further virologic and immunologic studies-of interest he has been previously noted to have a number of other abnormalities including significantly elevated IgM to CMV, and cortical and cerebellar atrophy on CT scan. Additional studies include repeat protein electropheresis, now with immunofixation, to identify viral antigens to which the patient's oligoclonal bands are directed. In our study of T lymphocyte subsets, several interesting findings emerged. Specifically, neuroleptics appeared to have no significant effect on the percentage of helper or suppressor T cells, either after acute or chronic administration. Helper T cells appear to be negatively correlated with age, duration of illness, and length of hospitalization in schizophrenic patients (all of whom were chronically hospitalized) and in those patients with Huntington's disease who had been chronically hospitalized (for all hospitalized patients correlation of length of hospitalization and helper T cells $r_S=-0.48$ $p < 0.01$). This fall in helper T cells (which maybe a consequence of prolonged hospitalization) might account for several anomolous immunologic findings in schizophrenia including isolated increases in IgM in the absence of increases in IgG (helper cells are needed to switch over from IgM to IgG) decreased natural killer cells (which depends in part on lymphokines, including interleukin 2, elaborated by helper cells), and reports of increased susceptibility to infection and decreased incidence of autoimmune disorders like rheumatoid arthritis in patients with schizophrenia. In addition, Oldstone has postulated that selective loss of helper cells might contribute to viral persistence in CNS (in models of murine lymphocytic choriomeningitis virus infection). Additional studies examining T lymphocyte subsets in CSF are planned, as are correlations of morphologic measures (such as CSF helper to suppressor T cell ratios) with functional measures (such as IgG index), to better delineate the role of both cell mediated and humoral immune dysregulation in schizophrenia.

Virus Neuron Interaction

Methods Employed: We had hoped to study in vitro the interaction of viruses with so called "luxury" function of nerve cells-such as neurotransmission-by measuring neurotransmitter

receptors in tissue culture prior to and following non permissive (that is, non-cytopathic) virus infection. In a pilot study, membranes from a number of neuroblastoma cell lines, including BJ1, BJ2, B103, and NIE115, lines which have allowed for a non-permissive infection by HSV1 (BJ1, BJ2, B103) or have demonstrated electrical response to dopamine and neurochemical evidence of dopamine uptake (NIE115) were examined for dopamine 2 receptor binding with ^{3}H-spiroperidol. In addition, we attempted to develop an in vivo model of non-permissive virus-neuron interaction by infecting Swiss Webster outbred mice with temperature sensitive mutant HSV (Glasgow 17 strain) and following these mice for behavioral changes (locomotion and stereotypy assessed on an optical digital sensor animal activity monitor), neuropathologic changes, and neurochemical changed catecholamines, their metabolites, and receptors).

Major Past Findings: Our attempt at developing an in vitro system to study virus-neuron interaction was disappointing. While saturable binding was found in NIE115 membranes this was not stereospecific and probably did not represent true receptors. Consideration is now being given to continue these studies in the neuroblastoma X Chinese hamster brain hybrid cell line NCB20, a newly characterized line which is the first, to our knowledge, to demonstrate dopamine 2 receptor binding and activity. In addition, studies have just been begun in collaboration with Dr. Richard Ziegler to grow primary fetal nerve cell cultures and monitor the electrophysiological and pharmacological effects of HSV infection. Furthermore we are still developing the temperature sensitive virus-murine model, including determining suitable multiplicity of infection, route of administration, age and strain of host, etc. and have yet to assess the behavioral and pharmacological effects of this non-permissive infection.

Significance to Mental Health Research: Despite a century of study the cause or causes of schizophrenia remain unknown. During the same century, our understanding of the nature of viruses and their potential role in disease has greatly expanded. It is most unlikely that a disorder like schizophrenia represents an acute encephalitis. Chronic neuropsychiatric disorders, however, may occur as the static consequences of acute encephalitis or as the progressive consequences of persistent infection. Moreover, we now appreciate that viral infections may be associated with both conventional and unconventional agents, agents which behave quite differently in standard virological and immunological assays. Furthermore, we have begun to appreciate that certain viruses, like the RNA containing retroviruses, can be intergrated in the host genome, only to become activated under appropriate conditions to produce seemingly spontaneous or inherited disorders. We have also seen that other viruses may effect CNS functioning, not only through cell death, but through alterations in brain monoamine metabolism or other "luxury" functions. Thus, a presumed viral infection is compatible with several aspects of schizophrenia: its delayed onset, protracted course, genetic basis, and association with neurotransmitter abnormalities. Viruses are attractive candidates for etiologic agents in at least some patients with schizophrenia. Schizophrenia, as a dementing illness of early onset, represents a significant public health problem, exacting an enormous cost in terms of lost capacity and productivity. Attempts to identify specific etiologic mechanisms, and thereby to develop specific primary preventive interventions are therefore of considerable importance. It must be noted, that chronic viral diseases (kuru, Creutzfeldt Jakob disease, progressive rubella panencephalitis, SSPE, PML) have only been identified by preliminary "leads" (appropriate animal models, epidemiological evidence of prior infection, electron microscopic visualization of viral structures) which have implicated a particular class of agents. In schizophrenia research such leads are notably lacking and study along several fronts, using a variety of assay techniques, may be necessary. In the process, we may also come to understand the ways in which neurovirologic and neuroimmunologic mechanisms influence a host of other psychiatric disorders.

Proposed Course of Study: This project began in 1978 with the first inoculation of brain tissue from schizophrenic patients into laboratory animals. It has involved ongoing collaborative efforts of psychiatrists and neurologists in the Neuropsychiatry Branch, virologists at the Laboratory of CNS Studies NINCDS, pathologists at the AFIP, and immunologists at Johns Hopkins University, and has been of benefit to all. Data analysis on earlier studies (such as attempts at transmission and identification of peripheral blood T lymphocyte subsets) is being completed, and additional studies, as outlined above, are underway under the direction of Dr. Kaufmann.

Publications:

Kaufmann, C.A., Stevens, J.R. and Torrey, E.F.: 1983 World Health Organization Symposium on Psychovirology. Arch. Gen .Psychiatry, 41:1184-1185, 1984.

Kaufmann, C.A., Kreek, M.J., Karoum, F. and Chuang, L-W.: Depression during methadone withdrawal: No role for beta-phenylethylamine. Drug Alcohol Depend. 13:21-29, 1984.

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Kaufmann, C.A., DeLisi, L.E., Torrey, E.F., Folstein, S.E. and Smith, W.J.: T lymphocyte subsets and schizophrenia. In Kurstak, Lipowski and Morozov (Eds.): Viruses, Immunity, and Mental Diseases. New York, Plenum Press, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01506-11 SMRP

PERIOD COVERED

October 1, 1984 to September 30, 1985

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)*

P.I.	H. Kageyama	Guest Research	LPP-NIMH
Other:	A. Guidotti	Section Chief	LPP-NIMH
	P. Panula	Visiting Fellow	LPP-NIMH
	E. Costa	Lab Chief	LPP-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:	PROFESSIONAL:	OTHER:
1.0	1.0	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.)*

In this project we intended to study the molecular mechanisms whereby the stimulation of opiate receptors produce a decrease of acetylcholine induced release of catecholamines from adrenal medulla cells. for this purpose we needed to develop a ligand capable of labeling nicotinic receptors in nervous tissues but since we have not yet been able to obtain a reliable ligand to study the binding properties of nicotinic receptors on neuronal cells, we could not continue this project.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 01514-13 SMRP

PERIOD COVERED
October 1, 1984 to September 30, 1985

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)*

P.I.	H. Kageyama	Guest Reseracher	LPP NIMH
Other:	A. Guidotti	Section Chief	LPP NIMH
	F. Tang	Guest Researcher	LPP NIMH
	T.T. Quach	Visiting Associate	LPP NIMH
	J.P. Schwartz	Research Chemist	LPP NIMN
	E. Costa	Lab Chief	LPP 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:	PROFESSIONAL:	OTHER:
1.0	1.0	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.)*

In this project we intended to explore how the increase in nuclear phosphorylation regulates the expression of gene coding for the induction of tyrosine hydroxylase and enkephalin like material in primary culture of cow adrenal medulla. We encountered several methodological problems related to phosphorylation in adrenal chromaffin cells in culture which together with the departure of Dr. Kageyama forced us to discontinue this project.

PHS 6040 (Rev. 1/84) GPO 904-917

Publication

Guidotti, A.: Enkephalin-like peptides as cotransmitters in splanchnic nerve and adrenal medulla. In: G. Delitala (ed): Opioid Modulation of Endocrine Function, New York, Raven Press, pp 247-253, 1984

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 01516-12 SMRP

PERIOD COVERED
October 1, 1984 to September 30, 1985

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)*

P.I.	A. Novelli	Guest Researcher	LPP-NIMH
Other:	A. Guidotti	Section Chief	LPP-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:	PROFESSIONAL:	OTHER:
1.2	0.7	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.)*

We tried to purify and characterize the benzodiazepine receptor subunit. Affinity chromatography studies with different benzodiazepines gave relatively good purification of the receptor but a very low yield. We tried purification with preparative SDS gel electrophoresis followed by HPLC reverse and exchange phase. Although preparative SDS gel produces a single band of proteins that is photolabeled with flunitrazepam, the HPLC separation with a different column and solvent was unsuccessful. Therefore the project was terminated.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 MH 01521-10 SMRP

PERIOD COVERED

October 1, 1984 to September 30, 1985

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 DIRP 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, skin, and ileum, a corresponding loss of somatostatin in spinal cord, ganglia and ileum, and no change in met^5-enkephalin-arg^6-phe^7 in any of these tissues. Adults exposed to anti-NGF show comparable losses of substance P and somatostatin content without a change in cell number in the sensory ganglia, as well as a loss of substance P in adrenal medulla and ileum.

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. In guinea pigs, which are most severely affected, the substance P content decreases 61% in spinal cord, 50% in ileum and 90% in sensory ganglia. Somatostatin decreases 53% in spinal cord and 65% in ileum, whereas met^5-enkephalin-arg^6-phe^7 does not change in any of these tissues. No change in these peptides occur in brain regions such as substantia nigra and striatum, in agreement with a probable lack of effect of NGF on brain neurons. In adult animals, in contrast, there is a depletion of the substance P and somatostatin content of sensory ganglia and spinal cord 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^5-enkephalin-arg^6-phe^7 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.

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 and how their synthesis is modulated by neurotrophic agents.

This project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 01525-09 SMRP

PERIOD COVERED
October 1, 1984 to September 30, 1985

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	DIRP	NIMH
	J.R. Naranjo	Guest Researcher	DIRP	NIMH
Others:	J.P. Schwartz	Research Chemist	DIRP	NIMH
	E. Costa	Lab Chief	DIRP	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:
3.3	2.5	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.)*

Direct measurement of mRNA levels can be made using cDNA probes and one can derive an estimate of peptide turnover by measuring the precursor mRNA, the precursor and the peptide itself. Treatment of bovine adrenal chromaffin cells with 8-Br-cyclic AMP results in an increase of both proenkephalin (PE) and tyrosine hydroxylase (TH) 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. Dexamethasone increases only PE mRNA and enkephalin peptides while reserpine depletes catecholamines and leads to TH induction while depleting PE mRNA and total enkephalin 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.

PHS 6040 (Rev. 1/84) GPO 904-917

Project Description:

Bovine adrenal chromaffin cells provide a model system in which to investigate regulation of the synthesis of co-transmitters, since enkephalin peptides are co-stored with catecholamines in their granules. We have utilized cDNA probes for proenkephalin (PE) and for tyrosine hydroxylase (TH) to measure changes in specific mRNA content in response to various pharmacological treatments. Exposure of the cells to 8-Br-cyclic AMP results in increased expression of both PE and TH mRNA 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 causes a dose-dependent increase of PE $mRNA_9$ as well as of enkephalin-related peptides, with a maximal 3-fold response occurring at 10^{-7} M. However, TH mRNA increases 50% only after 5 days of exposure to 1 μM dexamethasone and catecholamine content increases are less than 30%. Exposure of chromaffin cells to reserpine leads to a loss of catecholamines, a decreased content of PE mRNA, and conversion of HMW enkephalin precursors to the free peptides: the enkephalin peptide content therefore increases transiently before also decreasing. These studies demonstrate independent regulation of the synthesis of the co-transmitters in the adrenal medulla. 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.

The NG108 cell line contains both enkephalin peptides and a δ-opiate receptor, which has enabled us to study the effect of chronic exposure to an opiate agonist on synthesis of opioid peptides. Growth of the cells in the presence of etorphine leads to an increased content of PE mRNA by 5 days: the EC_{50} for this response is 10^{-9} M. Morphine has the same effect and the response is blocked by naloxone. Neither agonist causes long-term changes in cyclic AMP content, although increased cyclic AMP, produced by forskolin treatment, also increases PE mRNA. We plan to use this cell line to study the molecular mechanism by which activation of an opiate receptor can regulate opioid peptide synthesis.

We have developed a methodology with which to estimate changes in the dynamic state of neuropeptides and have applied it to pharmacological studies on rat brain. The method requires the measurement of precursor mRNA, of precursor proteins and of the biologically active peptides: the combination of these measurements can determine whether a drug affects a peptide by changing the amount of precursor mRNA, the rate of translation to or processing of the precursor, or the utilization of the peptide itself. 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. Studies using rats made tolerant to morphine show that there is no change in the PE system anywhere in the brain. POMC mRNA decreases 50% in the hypothalamus only, while β-endorphin levels remain normal, suggesting depressed functioning of the POMC system in morphine-dependent rats. Reserpine administration in vivo produces the same changes in adrenal medulla PE mRNA and enkephalin peptides as were seen with cultured chromaffin cells, supporting the hypothesis that whereas reserpine acts transsynaptically in the striatum to affect PE, it acts directly on the chromaffin cells of the adrenal medulla. 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, and to gain a better understanding of the mechanism of action of neuroactive drugs.

Publications:

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. 26: 255-260, 1984.

Mocchetti, I., Giorgi, O., Schwartz, J.P., and Costa, E.: A reduction of the tone of 5-hydroxytryptamine neurons decreases utilization rates of striatal and hypothalamic enkephalins. Eur. J. Pharmacol. 106: 427-430, 1984.

Mocchetti, I., Schwartz, J.P., and Costa, E.: Studies of brain neuropeptide dynamics utilizing cDNA probes: Pharmacological implications. In Caciagli, F. (Ed.): Physiological and Pharmacological Control of Nervous System Development. Amsterdam, Elsevier Press, 1984, pp. 77-80.

Costa, E., Guidotti, A., Hanbauer, I., Kageyama, H., Kataoka, Y., Panula, P., Quach, T.T., and Schwartz, J.P.: Adrenal medulla: Regulation of biosynthesis and secretion of catecholamines and enkephalins. In Usdin, E., Carlsson, A., Dahlstrom, A., and Engel, J. (Eds.): Catecholamines: Basic and Peripheral Mechanisms. New York, Alan R. Liss, Inc., 1984, pp. 153-161.

Schwartz, J.P., and Costa, E.: Hybridization approaches to the study of neuropeptides. Ann. Rev. Neuroscience, in press.

Mocchetti, I., Guidotti, A., Schwartz, J.P., and Costa, E.: Reserpine changes the dynamic state of enkephalin stores in rat striatum and adrenal medulla by different mechanisms. J. Neuroscience, in press.

Mocchetti, I., Schwartz, J.P., Costa, E.: Use of mRNA hybridization and radioimmunoassay to study mechanisms of drug-induced accumulation of enkephalins in rat brain structures. Mol. Pharmacol., 28:86-91 (1985).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: ZO1 MH 01531-08 SMRP

PERIOD COVERED

October 1, 1984 to September 30, 1985

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	DIRP	NIMH
Others:	J. Byrd	Guest Researcher	DIRP	NIMH
	T.T. Quach	Visiting Associate	DIRP	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:
1.4	1.4	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. 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 mRNA for this factor appears to hybridize with mouse β-NGF cDNA and is increased in pcd cerebellum. Entorhinal cortex lesions in rat stimulate production of a factor which can be assayed by its ability to support survival of chick embryo sensory and sympathetic neurons. The mRNA for the factor can be assayed by in vitro translation in an oocyte system.

PHS 6040 (Rev. 1/84)

GPO 904-917

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 neuronotrophic 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 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. Lesioned brains of newborn animals produce more factor activity than those of adults. mRNA has been isolated from control and lesioned brain. It is assayed for neurotrophic factor mRNA by injection into Xenopus oocytes: after 24 hr incubation, an extract is prepared from the oocytes and various dilutions tested in the biological assay. Lesioned brain mRNA is significantly more active than control brain mRNA. The mRNAs have been size-fractionated on sucrose gradients and individual fractions are being tested for neurotrophic activity, in order to purify the specific mRNA. In addition, Northern blots of control- and lesioned-brain mRNA have been probed with the β-NGF cDNA: a single band, smaller than the authentic NGF mRNA, was detected, with more in the lesioned brain. This may provide an additional method for assay of the factor. Once purified mRNA fractions are available, they will be used to prepare cDNA libraries, in order to clone the gene for the factor. In addition, we will prepare a cDNA library of messages specific to lesioned brain. This will be accomplished by preparing ^{32}P-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 have now prepared mRNA from the cerebellum, cortex and brain stem of control and pcd mice. On Northern blots, a single band is detected in the cerebellum approximately the same size as the β-NGF mRNA when probed with a -NGF cDNA: much less signal is present in either cortex or brain stem. We are preparing mRNA from brains of different-aged animals, to determine whether the mRNA detected increases in parallel with the increase in immunological activity. We will then clone the mRNA from a pcd cerebellum library in order to determine its sequence and properties.

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.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: ZO1 MH 01532-08 SMRP

PERIOD COVERED
October 1, 1984 through September 30, 1985

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)*

De-Maw Chuang, Chemist, Laboratory of Preclinical Pharmacology

COOPERATING UNITS *(if any)*
Carmine Coscia, St. Louis University Medical School

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:

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 frog erythrocyte agonist induced down regulation of β-adrenergic receptors (BAR) is associated with internalization of BAR binding sites; these internalized BAR sites are present in the 30,000 x g supernatant of erythrocyte homogenate. We found that these sites are associated with vesicular structures of more than 20×10^6 daltons. These BAR sites can be labeled by lipophilic ligands (dihydroalprenolol, cyanopindolol and hydroxybenzylpindolol) but not by hydrophilic ligands (CGP-12177 and isoproterenol), suggesting that these internalized BAR sites are present in inside-out endocytotic vesicles. In an attempt to elucidate the molecular events involved in BAR internalization, monoclonal antibodies against erythrocyte membrane components that react with BAR have been produced. Moreover we have investigated whether BAR internalization occurs in the CNS. Since clathrin-coated vesicles (CVs) have been implicated in both endocytotic and intracellular transport of a variety of receptors, we have isolated CVs from bovine brain and examined them for the presence of BAR and adenylate cyclase activities. Purified CVs were found to contain BAR binding sites assessed with ^{125}I-cyanopindolol as ligand in Sepharose column assays (Bmax=32±3 fmol/mg protein). The addition of GppNHp does not affect displacement of cyanopindolol binding to CVs by (-)isoproterenol, suggesting that these sites are uncoupled to the nucleotide regulatory protein. Moreover, these sites in CVs appear to be cryptic in nature, because ^{3}H-CGP 12177 fails to label BAR in CVs. In addition, adenylate cyclase activity can be detected in the pure preparation of CVs (24±0.5 pmol cAMP/mg/min). These activities are unaffected by GTP or isoproterenol + GTP. These data suggest that BAR and adenylate cyclase detected in brain CVs may be molecular entities undergoing endocytosis or intracellular transport.

PHS 6040 (Rev. 1/84) GPO 904-917

Project Description:

We have continued our studies on the molecular mechanisms of beta-adrenergic receptor BAR) desensitization. Our previous studies in the system of frog erythrocytes have provided the first evidence suggesting that down regulation of BAR induced by agonist exposure is associated with internalization of BAR binding sites; these internalized BAR sites are present in the 30,000 x g supernatant of erythrocyte homogenate. Recent data show that these sites are associated with vesicular structures of more than $20x10^6$ daltons (excluded by Sepharose 4B column chromatography). Moreover, these BAR sites can be readily labeled in a temperature dependent manner by lipophilic ligands such as dihydroalprenolol, cyanopindolol and hydroxybenzylpindolol but are labeled only with extremely low affinity by hydrophilic ligands such as CGP-12177 and isoproterenol. This suggests that these internalized BAR sites are present in inside-out endocytotic vesicles. In an attempt to obtain a better understanding of the molecular events involved in BAR internalization, we have attempted to produce monoclonal antibodies against BAR protein. Our initial attempt using crude detergent extract of purified erythrocyte plasma membranes as the antigen has resulted in the production of a monoclonal antibody whose antigenic determinant is a membrane component which reacts with BAR. Based on immunoblotting and immunoprecipitation, we have concluded that the antigenic protein (with Mr=43,000 and pI=6.2) is distinct from BAR sites and the alpha subunit of Ns (labeled with ^{32}P-ADP-ribose by cholera toxin) and have suggested that the monoclonal antibody is against an unidentified membrane component of the BAR-adenylate cyclase system. Our current strategies are to produce monoclonal antibodies to BAR using highly purified BAR as the antigen and to prepare anti-idiotype monoclonal anti-receptor antibody using a BAR antagonist as the immunogen. Immunohistochemical approaches in combination with biochemical studies will be used by us to unravel the details of each step involved in the catecholamine-induced BAR internalization and desensitization.

It is still unknown as to whether internalization of BAR occurs in the CNS. Since clathrin-coated vesicles (CVs) have been implicated in both endocytotic and intracellular transport of a variety of receptors, in collaboration with Dr. C.J. Coscia at St. Louis University, we have isolated CVs from bovine brain and examined them for the presence of BAR binding and adenylate cyclase (AC) activities. CVs were prepared from bovine forebrain as described by Pfeffer and Kelly. Microsomal pellets were subjected to linear D_2O/Ficoll gradient centrifugation and the resulting CVs enriched (60-80%) fraction applied to a controlled pore glass bead column to achieve further purification. The two major peaks of protein eluting from the column were monitored by electron microscopy and SDS-polyacrylamide gel electrophoresis. Peak II contained almost exclusively CVs, whereas Peak I which appeared in the void volume contained larger smooth vesicles and few CVs. BAR binding to Peaks I and II was assessed with ^{125}I-cyanopindolol (CYP) as ligand in sepharose 4B column assays. ^{125}I-CYP was found to bind specifically to sites in both peaks I and II with a Bmax of 28±4 and 32±3 fmol/mg protein, respectively. Binding of ^{125}I-CYP to both fractions was displaced by various BAR agents in a stereospecific manner. The addition of 50 M Gpp(NH)$_p$ did not affect the displacement of CYP binding to Peak II sites by (-) isoproterenol, whereas a significant right shift was noted when Peak I or a synaptic plasma membrane preparation (SPM) from bovine hippocampus was used. ^{3}H-CGP-12177, a hydrophilic BAR ligand, specifically bound to SPM and to a lesser extent to Peak I, but failed to label the BAR present in Peak II, suggesting that receptors present in CVs were cryptic in nature. We also found that AC activity could be detected in both Peaks I and II (spec. act.=21±0.6 and 24±0.5 pmol cAMP/mg/min, respectively). These activities were unaffected by GTP or isoproterenol + GTP. In contrast, a moderate stimulation of the

cyclase activity present in SPM was induced by these agents. Rechromatogaphy of Peak II on the glass bead column revealed that appreciable amounts of protein, CYP binding and AC activity were recovered in peak I; this change in chromatographic migration was facilitated by pre-exposure of CVs to 0.5 M Tris, a condition known to cause at least partial dissociation of clathrin from these vesicles. This suggests that at least some of the protein, as well as AC and CYP binding activities in Peak I were derived from CVs, possibly due to the loss of clathrin. Our results suggest that BAR and AC present in brain CV preparations might be molecular entities undergoing endocytotic or intracellular transport. We are currently investigating both possibilities.

It is well established that BAR in the CNS and peripherals can be up or down regulated when the neuronal sympathetic activities fluctuate in vivo. It has been suggested that this receptor modulation is of physiological and pharmacological importance. For example, down regulation of BAR induced by chronic antidepressant treatment is believed to be related to the therapeutic action. Up regulation of BAR associated with hyperthyroidism and propranolol withdrawal syndrome, and down regulation of BAR associated with hypothyroidism and hypoxia are most likely relevant to these disease states. The present study using the system of frog erythrocytes has suggested that internalization of BAR is a major mechanism for the receptor down regulation. Moreover, the detection and characterization of BAR in coated vesicles purified from bovine brain implies that internalization also takes place in the CNS. Thus, it seems reasonable to surmise that receptor down regulation is due to the acceleration of internalization whereas receptor up regulation may be the result of deacceleration of this metabolic event. Further study of the detailed mechanisms of BAR internalization may provide information for the design of drugs which may be used for the therapy of some diseases associated with abnormalties of BAR levels.

Publications:

Chuang, D.M.: A monoclonal antibody to a membrane component that interacts with beta-adrenergic receptor. J. Cyclic Nucleotide and Protein Phosphorylation Research, 110: 281-292, 1985

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01537-07 SMRP

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Trans-synaptic Control of the Ca^{2+}/phospholipid-dependent Protein Kinase.

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

PI: B.C. Wise

Others: F. Nicoletti
E. Costa

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:	PROFESSIONAL:	OTHER:
0.8	0.8	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.)*

The Ca^{2+}/phospholipid dependent protein kinase (protein kinase C) is an important intracellular mediator of the actions of extracellular signals that stimulate the breakdown of phosphatidylinositol to inositol phosphate and diacylglycerol. Diacylglycerol may be the second messenger, analogous to cAMP, that directly activates protein kinase C. The potential involvement of this enzyme in the functioning of adrenal chromaffin cells and cerebellar granule cells was the subject of this research project. Protein kinase C is present in bovine adrenal medulla and its constituent chromaffin cells in significant amounts compared to other tissue. In membranes and cytosol of the medulla, there are several specific endogenous substrate proteins for the enzyme. Treatment of primary cultures of chromaffin cells with phorbol esters, drugs which can substitute for diacylglycerol in the in vitro and in vivo activation of the enzyme, induce an apparent translocation of soluble enzyme activity to a membrane associated form. This translocation is specific for those phorbol esters that can activate the enzyme in vitro. Concomitant with the translocation of the enzyme activity is a significant phorbol ester induced release of catecholamines. Receptor-dependent activation of protein kinase C in cerebellar granule cells is also under study. Preliminary results indicate that glutamate, which stimulates phosphatidylinositol turnover in these cells, causes an approximate 20 to 30% increase in membrane-associated enzyme activity. The above studies indicate a potential prominent role for protein kinase C in chromaffin cells and cerebellar granule cells and that the receptor dependent activation of the enzyme may be manifested as a translocation of enzyme activity from cytosol to membrane.

PHS 6040 (Rev. 1/84)

GPO 904-917

Project Description:

The Ca^{2+}/phospholipid-dependent protein kinase (protein kinase C) is believed to be an intracellular mediator of the actions of extracellular signals that stimulate the hydrolysis of phosphatidylinositol to inositol phosphate and diacylglycerol. Diacylglycerol is an in vitro activator of protein kinase C and, hence may act as a second messenger intracellularly. The goal of this research project is to provide a clearer understanding of the role and physiological activation of this enzyme in intact primary cell cultures. Two well-characterized culture systems were studied: 1) bovine adrenal chromaffin cells and 2) rat cerebellar granule cells.

Chromaffin cells of the bovine adrenal medulla have been used as a model system to study the role of Ca^{2+}-dependent and cAMP-dependent protein kinases in the biosynthesis and release of catecholamines and opiate peptides. As a first step to study the role of protein kinase C in chromaffin cell function, a study on the possible presence of the enzyme and endogenous substrates for the enzyme in adrenal medullary tissue was initiated.

Measurement of enzyme activity with histone HI as an exogenous substrate demonstrated the presence of protein kinase C activity in subcellular fractions of the adrenal medulla. Enzyme activity was approximately equally distributed between the soluble (51% of total) and membrane (49% of total) fractions while the cAMP-dependent protein kinase was predominantly membrane bound (66% of total activity). Similar results were also found for both enzymes in chromaffin cells maintained in primary culture. The activity of the soluble protein kinase C of adrenal medulla was found to be about 50% of the enzyme level present in rat brain, a tissue previously shown to contain a very high enzyme activity, either when compared on a per gram of tissue or per mg of protein basis.

Studies on the phosphorylation of endogenous proteins indicate that the adrenal medulla possesses a prominant protein kinase C enzyme system. In the soluble fraction of the medulla, Ca^{2+} plus phosphatidylserine catalyzed phosphate incorporation into a number of substrates, which outnumbered substrate proteins for the cAMP-dependent enzyme. Prominent among the Ca^{2+} dependent substrate proteins were those of molecular weight 36, 17.7 and 16.5 kilodaltons of which the phosphorylation of the 36 and 17.7 kilodalton proteins showed an absolute dependency on both Ca^{2+} and phosphatidylserine and were not phosphorylated by the addition of Ca^{2+}/calmodulin, cAMP or cGMP. In membranes of the medulla only two specific substrate proteins (22.5 and 18.5 kilodaltons) were identified for protein kinase C. The Ca^{2+}/phospholipid-dependent phosphorylation was rapid with effects seen as early as 30 sec of incubation. These results taken together strongly implicate the Ca^{2+}/phospholipid-dependent phosphorylation system in multiple adrenal chromaffin cell functions.

Studies on the potential receptor-dependent activation of protein kinase C were carried out in adrenal chromaffin cells. One of the products of phosphatidylinositol hydrolysis is diacylglycerol which has been shown to be an in vitro activator of protein kinase C. A class of drugs, known as the tumor-promoting phorbol esters, can mimic the action of diacylglycerol on enzyme activity in vitro and in vivo, and so are useful tools to look at the consequences of in situ activation of protein kinase C. Treatment of chromaffin cells with TPA (1-100 ng/ml), a potent phorbol ester, led to a decrease in the soluble (maximal decrease of 95%), while concomitantly increasing the membrane associated (maximal increase of 191%), protein kinase C activity. The total cellular enzyme activity was unchanged. Thus, the results suggest an apparent translocation of soluble protein kinase C induced by TPA.

The effects of phorbol esters were rapid (near maximal effects seen as early as 5 min) and were biologically specific, that is, phorbol esters that activate the enzyme in vitro effected translocation, while those that are ineffective in vitro had no effect. TPA also produced a dose dependent (1-100 ng/ml) release of both norepinephrine and epinephrine from chromaffin cells, although the extent of the release was smaller than that seen with nicotine suggesting the involvement of additional mechanisms.

Several neurotransmitters and peptides were studied for their effects on protein kinase C translocation. No consistent effects of acetylcholine, carbachol, methacholine, GABA, substance P or opiate-like peptides were observed on the subcellular distribution of protein kinase C activity. Conclusions drawn from these negative results must be done with caution, since we know very little about the time course and extent of diacylglycerol (the postulated second messenger) formation in chromaffin cells.

The possible neurotransmitter-stimulated activation of protein kinase C was also investigated in primary cultures of cerebellar granule cells. These preliminary studies were carried out in collaboration with Dr. Nicoletti, who has demonstrated a large stimulation of phosphatidylinositol hydrolysis by glutamate in these cells. Glutamate treatment of granule cells caused about a 20 to 30% increase in membrane-associated enzyme activity. Although preliminary in nature, these results suggest that glutamate by stimulating diacylglycerol formation may activate protein kinase C with a consequent alteration of endogenous protein phosphorylation. Since studies in our laboratory demonstrate the presence of GABA, benzodiazepine, and chloride-channel interacting ligand binding sites in cerebellar granule cell cultures, it is of interest for future studies to address the effects of the activation of this receptor complex on protein kinase C activity and vice versa.

The above studies suggest potential prominent roles for the Ca^{2+}/phospholipid-dependent protein kinase in adrenal chromaffin cell and cerebellar granule cell functions. Since the adrenal medulla is important in the body's response to stress and, perhaps, its susceptibility to infections, continuation of this project may provide further insight into the normal and abnormal functioning of the adrenal gland. Very little is known about the mechanism of action of excitatory amino acid neurotransmitters in neurons. The studies using cerebellar granule cells may shed light on one possible intracellular mediator of glutamate action, namely protein kinase C and further studies should illuminate the role of protein kinase C in these cells.

Publications:

Wise, B.C. and Costa, E.: Ca^{2+} and phospholipid-dependent protein kinase activity and phosphorylation of endogenous proteins in bovine adrenal medulla. J. Neurochemistry, 45:227-234, 1985.

Gallo, V., Wise, B.C., Vaccarino, F., and Guidotti, A.: GABA and benzodiazepine-induced modulation of ^{35}S-t-butylbicyclophosphorothionate binding to cerebellar granule cells. J. Neuroscience, in press

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 01549-06 SMRP

PERIOD COVERED
October 1, 1984 to September 30, 1985

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:	PROFESSIONAL:	OTHER:
0.6	0.6	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 significantly decreases the number of specific high affinity recognition sites labelled by ^{3}H-imipramine and prevents the down regulation of α-adrenergic and of 5-HT$_2$ recognition sites elicited by repeated daily injections of imipramine (IMI) and desmethylimipramine (DMI). Moreover after daily injections of IMI and DMI for 2-3 weeks the max of ^{3}H-IMI binding is decreased while the Vmax of ^{3}H-5-HT uptake in hippocampus slices is increased. These findings suggest: 1) a relevant pharmacological role of the ^{3}H-IMI recognition site in mediating some of the neurochemical effects elicited by chronic IMI treatment 2) a possible physiological role of the ^{3}H-IMI recognition on sites in modulating the gain of the 5HT reuptake and thereby the 5HT synaptic transmission; 3) the existence of a putative endogenous ligand (endacoid) for the ^{3}H-IMI binding site which physiological modulates the ^{3}H-5HT reuptake. A nonpeptide thermostable putative endacoid which inhibits in a dose-dependent manner. ^{3}H-IMI binding and 5HT uptake very partially purified from rat brain. HPLC retention time and other chemical physical properties differentiate it from 5HT and a series of indole-alkyl devivatives including tryptoline, 5-hydroxy and 5-methoxy-tryptoline. Crude synaptic membrane from rat brain contain also specific and high affinity reception sites for ^{3}H-Mianserin an atypical antidepressant. The ^{3}H-Mianserin reception sites appear to be different from the 5HT$_2$ recognition sites labelled by ^{3}H-Ketausei: 5HT axon terminals lesion and pharmacological treatments elicita different modifications upon the two binding sites. The ^{3}H-Mianserin recognition site is proposed to be part of the supramolecular organization of the 5HT$_2$ receptor complex are involved in the modulation of the seronergic synaptic transmission.

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 α-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 α-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 α-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 Bmax of ^{3}H-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 (Vmax) 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 ^{3}H-imipramine binding sites is decreased by repeated imipramine injections. Our data support the possibility that the sites where ^{3}H-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 ^{3}H-imipramine binding sites from rat brain which, in a dose dependent manner, inhibits 5-HT uptake and displaces ^{3}H-imipramine binding. We have tested this putative endocoid on ^{3}H-flunitrazepam, ^{3}H-mianserin, ^{3}H-dihydroalprenolol binding and none of these ligands could be displaced by the inhibitor of ^{3}H-imipramine binding. The endocoid extracted from rat brain appears to have a molecular weight of 1,800 daltons, it is polar, 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 ^{3}H-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- -carboline, harmol, harmaline, harmine, norharmane, harmane, 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 ^{3}H-imipramine binding and 5-HT uptake (IC_{50}s 0.8 and 0.2 M and 0.6 and 0.8 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. In rat, its whole brain content is not affected by a pretreatment of the rat with reserpine or P-chlorophenylalanine (p-CPA) to defect catecholamines and serotonin stores or to inhibit 5HT synthesis, respectively. However a lesion of 5HT axon terminals by 5, 7-dihydroxy-tryptamine given intracerebroventricularly decreases, while a preloading of the rats with L-tryptophan ethyl ester increases the whole brain endocoid contact. It appears to be unevenly distributed in various rat brain structures: the richest being c. stratium hippocampus cerebral cortex dieucyphalon brain stem cerebellum, hypothalamus, olfactory bulb. The identification of the nature of this futative endocoid would prompt us with new information on the supramolecular organization of the serotonin reuptake system, on the multiplicity of signals at the synaptic level and eventually would enable us to measure it in biological fluids of patients suffering from various kinds of affective disorders. This compound may turn out to be an important marker for dixases in which the serotonergic sytem is involved. Preliminary results obtained in GC/MS seem to favor the possibility that a sort of tricyclic structure with the following elemental composition: $C_{24}H_{17}N_3O_4$ might be present in the rat brain extract that contains the putative endacoid for ^{3}H-imipramine binding.

Crude synaptic membranes of rat brain contain specific high affinity binding sites for an atypical antidepressant, mianserin. It was previously suggested that ^{3}H-mianserin labels 5-HT_2 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 ^{3}H-mianserin binding sites are increased following lesion of 5-HT axons with 5,7-dihydroxytryptamine whereas the binding characteristics of ^{3}H-ketanserin remain unchanged. Moreover repeated daily injections of imipramine decrease the specific binding of ^{3}H-ketanserin but fail to affect the binding of ^{3}H-mianserin to crude synaptic membranes prepared from rat hippocampus or cortex. The ^{3}H-mianserin and ^{3}H-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 Bmax of ^{3}H-ketanserin binding, while does not affect the ^{3}H-mianserin binding kinetic characteristics. Mianserin can decrease the Bmax 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 ^{3}H-mianserin or ^{3}H-ketanserin binding. Only after 7 days of repeated daily injections ketanserin down-regulates ^{3}H-ketanserin recognition sites while it does not change the ^{3}H-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-HT_2 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_2 axon that acts through the ^{3}H-mianserin binding site; the other labeled by ^{3}H-ketanserin or ^{3}H-spiroperidol which is called 5-HT_2 receptor and is modulated by the effector produced by 5-HT axons that binds on ^{3}H-mianserin binding site. We are currently trying to verify this model by studying the interactions between the ^{3}H-ketanserin and the ^{3}H-mianserin binding sites and by trying to isolate the possible endogenous effector for the ^{3}H-mianserin site.

Moreover, we investigated 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 ^{3}H-ketanserin and ^{3}H-mianserin binding sites in the frontal cortex and hippocampus but do not modify the binding characteristics of ^{3}H-5-HT$_1$ receptor recognition sites. The time course of the modifications of ^{3}H-mianserin and ^{3}H-ketanserin binding after iprindole show that while the Bmax of ^{3}H-ketanserin binding is decreased already after 4 days of treatment, the Bmax of ^{3}H-mianserin binding is decreased only after 1 week of treatment. Moreover while the decrease of ^{3}H-ketanserin binding elicited by repeated imipramine injections is prevented by a selective lesion of the 5-HT axon terminals, the decrease of ^{3}H-ketanserin and ^{3}H-mianserin binding evoked by iprindole are not sensitive to serotonergic denervation. Since "in vitro" experiments have shown that iprindole can displace the ^{3}H-mianserin bound to its recognition sites with an IC_{50} value which is lower than that necessary to displace other ligands one could surmise that iprindole may be acting directly on the postsynaptic ^{3}H-mianserin and/or ^{3}H-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 α-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 α-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 ^{3}H-mianserin, ^{3}H-ketanserin and ^{3}H-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 α-adrenergic receptor function.

We have also studied the pharmacological profile of maprotiline which is a drug of repeatedly claimed clinical efficacy. Its most peculiar phamacological property is that it potently and selectively inhibits the reuptake of norepinephrine (NE). Despite this short term and "in vitro" effect, when maprotiline was given daily for 3 weeks, i.p. to rats the activity of the betadrenoceptor coupled a cAMP generating system and the beta adrenoceptor number was not reduced. This was at variance with what one would expect following continuous blockage of the NE reuptake and chronic exposure of the postsynapsis to increased amounts of endogenous agonist. Furthermore, 3 weeks of treatment with maprotiline did not increase the number of $5HT_2$ recognition sites or of ^{3}H-miaserin recognition sites, although maprotiline has a reasonably high affinity for these two recognition sites in vitro. However, the same treatment schedule did elicit a decrease in ^{3}H-flunitrazepam H-flu and ^{3}H-beta-carboline ethyl ester (^{3}H-B-cee) binding in the hypothalamic and hippocampal membranes. These effects (decreased Vmax for ^{3}H-flu and decreased affinity for ^{3}H-Bc binding) do not appear to be related to changes in endogenous GABA levels in the membrane preparation. These results raise several points: 1) The down regulation of the beta adrenoceptor linked cAMP generating system following repeated daily antidepressant treatments does seem to depend purely on the increase in synaptic levels of endogenous transmitters; 2) there appears to be more and more exceptions to the rule that all antidepressants decrease the beta adrenoceptor function upon chronic treatment; and 3) the brain benzodiazepine/GABA-receptor sytem is likely to be involved in the long term action of antidepressants.

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.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 01552-05 SMRP

PERIOD COVERED

October 1, 1984 to September 30, 1985

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)*

P.I.	M. Ferrero	Guest Researcher	LPP NIMH
Other:	A. Guidotti	Section Chief	LPP-NIMH
	E. Costa	Lab. Chief	LPP 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:	PROFESSIONAL:	OTHER:
1.0	1.0	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.)*

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 drugs belong to benzodiazepine (anxiolytic) or beta-carboline (anxiogenic) type of ligands we developed a behavioral animal model that predicts the anxiogenic and anxiolytic potency of a drug. In this test the imidarobenzodiazepine, RO 15 1788 acts like an inert antagonist of both beta-carboline like or benzodiazepine like drugs. However, in animals in which GABAergic transmission is reduced by the administration of small doses of the glutamic acid decarboxylase inhibitor isoniazid, RO 15 1788 became a weak beta carboline like agent. These data suggest that multiple chemical signals are operative in the expression of GABAergic transmission and that full understanding of this multiplicity require pharmacological manipulation at GABAergic synapses.

PHS 6040 (Rev. 1/84) GPO 904-917

This project has been terminated.

Publication

Costa, E., Ferrari, M., Ferrero, P. and Guidotti, A.: Multiple signals in GABAergic transmission: pharmacological consequences. Neuropharmacology 23: 989-991, 1984

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01553-05 SMRP

PERIOD COVERED

October 1, 1984 to September 30, 1985

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:	PROFESSIONAL:	OTHER:
1.0	1.0	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.)*

The reason this project was terminated is because the principle investigator has left.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: ZO1 MH 01555-05 SMRP

PERIOD COVERED
October 1, 1984 through September 30, 1985

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)*

P.I.:	B. Mellstrom	Visiting Fellow	DIRP	NIMH
Others:	H.-Y.T. Yang	Section Chief	DIRP	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:	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.)*

We have previously shown that Met^5-enkephalin-Arg^6-Phe^7 (YGGFMRF) is metabolized by dipeptidyl carboxypeptidase and aminopeptidase. In this study we have searched for an effective enzyme inhibitor that is capable of crossing the blood brain barrier and blocking the inactivation of YGGFMRF by the dipeptidyl carboxypeptidase in CNS. HOE 498 was found to be a potent inhibitor of this enzyme. This compound was tested for its ability to cross the blood brain barrier. The presence of HOE 498 in rat cerebrospinal fluid (CSF) was demonstrated after I.V. or I.P. injection. The presence of HOE 498 in brain was also demonstrated by the increased recovery of YGGFMRF injected into the brain of rats pretreated I.P. with the inhibitor. Further-more, the recovery of YGGFMRF released from striatal tissue slices was increased in the presence of HOE 498. Whether this inhibitor can potentiate the analgesic effect of YGGFMRF still remains to be studied.

PHS 6040 (Rev. 1/84) GPO 904-917

Project Description:

It was previously shown that Met^5-enkephalin-Arg^6-Phe^7(YGGFMRF) is metabolized by a dipeptidyl carboxypeptidase and aminopeptidase. The recovery of YGGFMRF released by substance P into the subarachnoidal space is increased by infusion of the dipeptidyl carboxypeptidase inhibitor, captopril. However, captopril does not readily cross the blood brain barrier and the aim of this study is to search for effective enzyme inhibitors which are capable of crossing the blood brain barrrier and blocking the YGGFMRF inactivation by the dipeptidyl carboxypeptidase in CNS.

A microsomal preparation from mouse striatum was used as the enzyme source and enzyme activity was assayed by quantitation of the enzymatic product, Met^5-enkephalin, by HPLC. Potential inhibitors provided by Dr. Blumberg, Revlon and Hoechst were tested for their inhibitory effect in vitro. The most potent compound was found to be HOE 498 diacid, 2-(N(S)-1-ethoxycarbonyl-3-phenylpropyl)-L-alanyl)-(1S, 3S, 5S)-2-azabicyclo(3.3.0) octan-3-carbonic acid, with an IC_{50} value of 0.8 nM. In order to test whether this compound and its prodrug HOE 498 ester are capable of crossing the blood brain barrier, rats were tested with the drug I.V. or I.P. and then the CSF was drawn and tested for the presence of the inhibitors. After I.P. or I.V. injection of 0.01 mg/kg HOE 498 diacid, the enzyme inhibitor was detected in the CSF. Further-more dipeptidyl carboxypeptidase activity, which is present in CSF, was decreased in a dose dependent manner after I.P injection of HOE 498. To show the presence of the enzyme inhibitor in the brain, HOE 498 diacid was injected into the caudate followed by injection of YGGFMRF into the same brain area. The recovery of injected I.P. and brain tissue was increased in HOE 495 pretreated rats compared to saline injected controls. The effect of the inhibitor on the recovery of YGGFMRF relesed by 47 mM KCL from striatal slices was also tested. YGGFMRF released into the incubation medium was measured by RIA. In the presence of HOE 498 diacid, the recovery of YGGFMRF was increased compared to when only bestatin was added to the incubation medium. The results suggest that HOE 495 is a potent inhibitor of YGGFMRF inactivating enzyme, the dipeptidyl carboxypeptidase, and is capable of crossing the blood brain barrier with doses used in this study.

The significance of this study to the biomedical research is that the availability of this potent enzyme inhibitor may be very useful in studying the functional role of the opioid peptide YGGFMRF in the CNS, and also the physiological release of YGGFMRF.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01558-04 SMRP

PERIOD COVERED

October 1, 1984 through September 30, 1985

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

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NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 2032

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.1	1.1	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 reason this project was terminated is because the principle investigator has left.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 MH 01559-04 SMRP

PERIOD COVERED

October 1, 1984 through September 30, 1985

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)*

Hsui-Ying Yang, Ph.D., Section Chief, Laboratory of Preclinical Pharmacology

Dr. Erminio Costa, M.D., Chief, Laboratory of Preclinical Pharmacology

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Neuropeptide

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

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.)*

Previously, we have presented several lines of evidence suggesting that Phe-Met-Arg-Phe-NH_2 -like (FMRF-NH_2-like) peptide(s) in mammalian CNS may have modulatory role in opiate antinociception. FMRF-NH_2 was a neuropeptide of clam origin, however, presence of FMRF-NH_2-like peptides, which are distinct from the tetrapeptide FMRF-NH_2, in mammalian CNS is now well known. In order to further explore the possible role of endogenous FMRF-NH_2-like immunoreactivity in opiate antinociception, we have decided to chemically characterize the mammalian FMRF-NH_2 like peptides. Two novel peptides, which are immunoreactive to FMRF-NH_2 antiserum, have been purified to homogeneity from bovine brain and their amino acids sequences determined. Furthermore, these two peptides (octapeptide and octadecapeptide) were synthesized and biological activities were tested in rats. Both peptides, when injected intraventricularly, were found to decrease tail flick latencies of rats. One of the peptides, octapeptide, was found to attenuate the analgesia induced by morphine significantly. The molecular mechanism underlying this antiopiate effect of the novel mammalian FMRF-NH_2 like peptide is under current investigation.

Project Description:

The cardioexcitatory peptide, Phe-Met-Arg-Phe-NH_2 (FMRF-NH_2), was originally isolated from the ganglia of the venus clam, Macrocallista nimbosa by Price and Greenberg in 1977 (Science 197:670,1977). Subsequently, FMRF-NH_2 like immunoreactivity was detected in mammalian CNS by the antiserum raised against FMRF-NH_2. In searching for the biological role of FMRF-NH_2 like material in mammalin CNS, we have previously presented several lines of evidence indicating that endogenous FMRF-NH_2 like peptide may have a role in opiate mediated analgesia. Because of this, in this study we have decided to isolate the FMRF-NH_2 like peptide from bovine brains and study their biological activities. Two peptides which are immunoreactive to the antiserum raised against FMRF-NH_2 were purified to homogeneity by an immunoaffinity column chromatography and successive steps of reversed phase HPLC under various different chromatographic conditions. These two purified peptides were sequenced by Applied Biosystems using a gas-phase sequencer. Based on the results of the sequence analysis and the specificity of the antiserum used for detection, the amino acid sequence of the two peptides were determinedto be 1) Ala-Gly-Glu-Gly-Leu-Ser-Ser-Pro-Phe-Trp-Ser-Leu-Ala-Ala-Pro-Gln-Arg-Phe-NH_2(Ala-18-Phe-NH_2) and 2) Phe-Leu-Phe-Gln-Pro-Gln-Arg-Phe-NH_2 (Phe-8-Phe-NH_2). Two peptides, with the amino acid sequences as shown above, were synthesized by Peoninsula Laboratories Inc. and their biological activities were studied. Both peptides, Ala-18-Phe-NH_2 and Phe-8-Phe-NH_2 were found to decrease the basal tail flick lantency of the rat when injected intraventricularly. The effect was short lasting and the octapeptide, phe-8-phe-NH_2, was more active than the octadecapeptide, Ala-18-Phe-NH_2. The peptide, Ala-18-Phe-NH_2, decreased the tail flick lantency at 20 μg but not at 10 μg. The peptide, Phe-8-Phe-NH_2, was found to be still active at 5 μg which was the lowest dose tested. The peptide, Phe-8-Phe-NH_2 caused barrel rotations in some rats at 20 μg and in all rats at higher doses. Phe-8-Phe-NH_2, (5 μg) when injected intraventricularly prior to the drug, decreased the analgesia elicited by morphine significantly. The molecular mechanism underlying this antiopiate iffect of the peptide Phe-8-Phe-NH_2 is still unclear and is under current investigation. Whether Phe-8-Phe-NH_2 participates in development of opiate tolerance will be explored.

The significance of this study on biomedical research is that the characterization of a novel peptide with antiopiate activity may add a new direction for studying the development of opiate tolerance.

The proposed course of the study are 1) to develop antibodies against Phe-8-Phe-NH_2 and Ala-18-Phe-NH_2 and then to study their distribution 2) to characterize receptors for these two new peptides, 3) to determine whether these two new peptides have effect on opiate receptor and 4) to determine whether there is alteration in the content of FMRF-NH_2 like peptides in morphine tolerant rats.

Publications:

Yang, H-Y.T., Tang, J., Iadarola, M., Panula, P., and Costa, E.: Are Phe-Met-Arg-Phe-NH_2 immunoreactive peptides endacoid modulating opiate antinociception? in proceedings of the First International Symposium on Endacoids ed Lal, H. (Raven Press, New York), in press.

Yang, H-Y.T., Fratta, W., Majane, E.A. and Costa, E.: Isolation, sequencing, synthesis and pharmacological characterization of two new brain neuropeptides modulating the action of opioids. Proc. Natl. Acad. Sci. USA, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: ZO1 MH 01561-04 SMRP

PERIOD COVERED
September 1, 1985 through October 31, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Neuropeptides and Somatosensory Processing

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

Michael J. Iadarola, Staff Fellow, Laboratory of Preclinical Pharmacology

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Section on Neuropeptide

INSTITUTE AND LOCATION

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 concerns the role of spinal cord peptide-containing neurons in sensory processing. A model of chronic peripheral inflammation has been developed in order to relate changes in the dynamic state of enkephalin, dynorphin (opioid) and cholecystokinin neurons to prolonged abnormal afferent input. A three-fold increase in dorsal spinal cord dynorphin content (dynorphin A 1-18 and 1-17) occurs by six days of inflammation and significant increases are observed as early as 3 days. The immunoreactivity materials have been identified by HPLC and gel chromatography in both control and treated conditions. Maintenance of the inflammation up to 14 days maintains the increase in dynorphin. The increase is specific to dorsal horn where sensory processing occurs, and is confined to spinal segments which subserve hindpaw sensation; no alteration of cervical cord dynorphin is observed. At the same time, no significant alteration in dorsal cord enkephalin (met^5-enkephalin-arg^6-gly^7-leu^8) or CCK content is obseved. However, hybridization of the total dorsal cord RNA with a proenkephalin specific cDNA probe reveals a sharp increase in enkephalin mRNA content at 24 hours with return to near normal by three days. In periaqueductal grey a 40-70% increase in enkephalin content occurs by day of inflammation. No change occurred in dynorphin or CCK in this and several other brain regions.

These data suggest an acute by transient activation of spinal enkephalin neurons and a delayed but sustained activation of spinal dynorphin neurons in response to chronic pain. Brain enkephalin pain pathways also respond to the chronic stimulus. The significance of these studies is that they provide a model in which to study the regulation of opioid neurons and a new framework from which to evaluate the role of multiple spinal opioid systems in control of chronic pain as encountered in arthritis and cancer. Further elucidation of the pivotal role of the spinal dynorphin system may provide a new avenue for the pharmacotheraphy of the chronic pain state as well as insights into chronic opioid abuse and tolerance.

PHS 6040 (Rev. 1/84) GPO 904-917

Project Description:

This project has been concerned with neurochemical characterization of a model of chronic peripheral inflammation/pain on spinal cord and brain peptidergic systems. The current focus on pathological states has evolved from earlier, simplier biochemical studies of opioid and CCK systems in cord and brain. Several new and important observations have been made with this model. Chief among them is the dynorphin content in dorsal cord ipsilateral to the affect limb undergoes a three fold increase. This increase has been observed with two representative end products of the prodynorphin precursor:dynorphin A-18 and dynorphin A 1-17 (we have raised antibodies and developed RIAs for both peptides). The increase occurs with three days of the onset of inflammation and is maximal by day 6. If the inflammation is sustained for 14 days the increase in dynorphin is also sustained. The immunoreactive materials in the crude extract have been chromatographically identified by HPLC and gel filtration in both control and treated conditions. The increase is restricted to dorsal cord, where sensory processing occurs, no change is found in ventral cord although basal dynorphin levels are very low here. The increase is segmentally specific: if one foot is treated only the dorsal cord that subserves sensation from that limb undergoes an increase; no change occurs in the contralateral lumbar dorsal cord or cervical cord from either the control or treated side. In fact, levels of dynorphin 1-8 in the affected regions are identical to those in untreated rats.

Immunocytochemical localization of the dorsal horn neurons involved has shown them to be small pear shaped cells with a prominent dorsally projecting dendrite(s). The immunoreactive somata are arranged in a discrete band situated in lamina III near the border of lamina II. Immunoreactivity terminals are most concentrated in substantia gelatinosa and presumably arise from the axons of the above mentioned cells. terminal immunoreactivity also appears to be increased in the dorsal horn corresponding to the inflammed limb in comparison to the dorsal cord from axon treated limb.

In contrast to the substantial changes in spinal dynorphin content, no significant alteration occurred in the content of the proenkephalin derived peptide met^{5}-enkephalin-Arg^{6}-gly^{7}-leu^{8} (MERGL) at any time point examined (3, 8, or 14 hrs or 1, 1.5, 3, 8, or 14 days). However, preliminary evidence obtained by measuring proenkephalin mRNA with cDNA hybridization to dorsal cord RNA blots revealed a 2-fold increase at 24 hrs. These data suggest an initial but transient activation of proenkephalin neurons in response to chronic inflammation. We are in the process of verifying this observation a second time and shall include some earlier time points. In addition, we shall measure the dynorphin RNA to determine whether an increase occurs in prodynorphin synthesis in conjunction with the increase in content. These future studies are necessary to determine if the increase in tissue peptide content is due to an increase in the synthesis and release or due to an inhibition of activity and a decrease in release.

Additional studies in brain reveal increases in enkephalin content of two brain regions, CCK and dynorphin were measured in these same samples (periaqueductal grey, hypothalamus, caudae, septum and substantia nigra). MERGL content was elevated in periaqueductal grey, an area known to be important in opiod analgesia. This observation has been verified and occurs with either one or both feet inflammed. The other area that showed an increase in MERGL content was the substantia nigra. The reason for this nucleus being affected is unclear but it may reflect an activation of the amygdala since nigral enkephalin apparently derives from an amygdaloid projection. The selectivity of the change in enkephalin is underscored by the lack of alteration in MERGL content in septum, caudate or hypothalamus and that the dynorphin and CCK content did not change in any of the five regions.

Our results so far suggest a widespread activation of the CNS proenkephalin system to chronic pain. The activation appears transient in spinal cord and may be more sustained in brain. The prodynorphin system displays a specific and prominent alteration at the spinal level but little alteration in brain. No change in CCK content has been detected as yet in either brain or cord.

The significance to biomedical research lies in our demonstration of a previously undisclosed role for dynorphin at the spinal level in the CNS response to chronic peripheral inflammation. Furthermore, proenkephalin is altered in several brain sites such as the periaqueductal grey and the substantia nigra. The latter sites may provide an index of the emotional, affective response to pain and demonstrate that several levels of the CNS are involved in the response to chronic pain. These findings may eventually allow for a more effective targeting of pharmacotheraphy to the dynorphin class of spinal opioid neurons in the treatment of arthritis and cancer pain as well as in understanding mechanisms of addiction and tolerance to chronic opioid abuse.

References:

Iadarola, M. J., Panula, P., Majane, E. A. and Yang, H.-Y. T.: The opioid cotapeptide met^5-enkephalin-arg^6-gly^7-leu^8: characterization and distribution in rat spinal cord. Brain Research 30:127-134, 1985.

Iadarola, M. J., Fanelli, R. J., McNamara, J. O. and Wilson, W. A.: Comparison of the effects of diphenylbarbituric acid, phenobarbital pentobarbital and secobarbital on GABA-mediated inhibition and benzodiazepine binding. J. Pharmacol. & Exp. Ther. 232:127-133, 1985.

Iadarola, M. J., Shin, C., McNamara, J. O. and Yang, H.-Y. T.: Changes in enkephalin dynorphin and cholecystokinin in hippocampus and substantia nigra after amygdala kindling in rats. Brain Research, in press.

Iadarola, M. J. and Yang, H.-Y. T.: Relationship between dopamine and cholecystokinin in rat forebrain: a biochemical study of co-localization. J. Neurochem., in press.

Nicoletti, F., Meek, J. L., Iadarola, M. J., Chuang, D. M., Roth, B. L. and Costa, E.: Coupling of inositol phospholipid metabolism with excitatory amino acid recognition sites in rat hippocampus. J. Neurochem., in press.

Nicoletti, F., Barbaccia, M. L., Iadarola, M. J., Pozzi, O. and Laird, H.: Abnormality of alpha$_1$-adrenergic receptors in frontal cortex of epileptic rats. J. Neurochem., in press.

Kleinman, J. E., Hong, J., Iadarola, M. J., Govoni, S., Gillin, J. C.: Neuropeptides in human brain - postmortem studies. Prog. in Neuropsychopharmacol. & Biol. Psychiat., 9:91-95, 1985

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01567-02 SMRP

PERIOD COVERED

October 1, 1984 through September 30, 1985

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

Role of Synaptosmal 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)*

F. Vaccarino, Guest Researcher, Laboratory of Preclinical Pharmacology

A. Guidotti, Section Chief, LPP; E. Costa, Laboratory Chief, LPP

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:	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.)*

GABA-modulin (GM), a basic protein which regulates GABA receptor binding to post synaptic receptors, has been purified from rat brain synaptosomes and biochemically characterized. Although GM presents some similarities with small myelin basic protein (SMBP), it is biochemically and functionally distinct from any known myelin basic protein.

To locate GM in brain, monoclonal and polyclonal antibodies have been produced; one polyclonal antiserum was 4000 - fold specific for GM as opposed to SMBP and detected a single immunoreactive molecular species in rat brain. Granule cells in primary culture, which express the GABA-BZ-CI - receptor/ionophore complex, also contain a protein which is identical by immunological, biochemical and functional criteria to GM isolated from rat brain synaptosomes.

PHS 6040 (Rev. 1/84) GPO 904-917

Project Description:

GABA-modulin (GM) is a basic protein which has been shown to down-regulate by an allosteric mechanism the high-affinity binding of GABA to post-synaptic receptors (Guidotti et al, PNAS, 79:6084, 1982) - GM has been extracted and purified from the synaptosomal membrane fraction of rat brain, where it is highly concentrated (approximately 3μg/mg protein). Although GM presents similarities to the class of highly basic protein present in myelin, in particular to the small myelin basic protein (SMBP), GM is selectively located in synaptosomes and differs from SMBP for molecular weight, amino acid composition, amino acid sequence and peptide mapping. Since GM has been isolated from a synaptosomal fraction derived from brain homogenate, it was necessary to demonstrate its coexistence with GABA receptors in a population of isolated neurons as a prerequisite of studying the physiological role of this protein in brain. Primary cultures enriched in cerebellar interneurones, predominantly granule cells, were prepared from the cerebellum of neonatal rats; these cultures undergo both morphological and biochemical differentation and contain less than 5% of asxtrocyte and oligodendrocyte cells (GALLO et al, PNAS 79:7919, 1982; KINGSBURY et al, Develop Br. Research 10:17, 1985 LEVI et al, Develop Br. Research 10:227, 1983). Granule cells in primary culture represent an excellent model for studying the GABA-BZ-Cl-receptor/ionophore complex and its regulation. In fact, they express GABA receptors, benzodiazepine receptors and Cl-channel-associated receptors, as detected by ^{3}H Muscimol, ^{3}H Flunitrazepam and ^{3}S-butylbicyclophosphorothianate respectively. 3H Muscimol binding carried out under physiological conditions and on the surface of undisrupted cells demonstrated a Bmax comparable to rat cerebellar membrane preparations and was up-regulated by benzodiazepines. In order to detect the presence of GM in cultures of granule cells, monoclonal and polyclonal antibodies have been prepared. For the preparation of monoclonal antibodies, NZB/N mice were immunized with purified GM and their lymphocytes were fused with the P3 X 63 myeloma cell line. Several clones were selected for the secretion of antibodies reactive against GM, however these antibodies completely cross-reacted with myelin basic proteins except one, which exhibited a 100-fold specificity for GM. The polyclonal antiserum, prepared in rabbits, was routinely preabsorbed with myelin basic proteins and, after preabsorption, maintained a relatively high affinity for GM (50% displacement of tracer bound with 2 pmol of protein) but showed a 4000-fold specifity for GM. Thus, this antiserum was used to study GM location in granule cell cultures.

Immunohistochemical studies demonstrated a very strong GM-like immunoreactivity located in the cell bodies and along cell processes of all granule cells. No staining was evident in glial cells.

To characterize the GM-like immunoreactive material, a crude acetic extract of either rat brain synaptosomes or granule cell cultures was fractionated through reverse-phase HPLC and each fraction analyzed by RIA. The profile was identical in both cases and showed a single peak of immunoreactivity, co-eluting with purified synaptosomal GM.

The GM-like immunoreactive material, purified from granule cell cultures by a standard biochemical procedure, resulted to have identical retention time on HPLC as well as identical molecular weight on SDS-PAGE with respect to GM purified from synaptosomes. In addition, amino acid analysis of granule cells derived GM revealed an amino acid composition very similar to GM purified from synaptosomes.

Thus, granule cells contain a protein which appears to be identical, by immunological and biochemical criteria, tosynaptosomal GM. This protein isolated from granule cells inhibits 3H muscimol binding with an I.C.30 of 0.5 μM, which corresponds to the inhibitory potency of synaptosomal GM.

The presence of GM in a homogeneous population of neurons like granule cells, which express GABA receptors, receive a strong GABA input and do not utlize GABA as a neurotransmitter, is not only a definite proof of the neuronal location of GM, but it is highly indicative of a post-synaptic location of this protein. Given the availability of a specific antibody probe, additional studies can be performed which may reveal the exact mode of action of GM and may lead to new concepts regarding modulation of receptor function by endogenous membrane proteins in brain.

Publications:

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., 44: 278, 1985.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01569-03 SMRP

PERIOD COVERED

October 1, 1984 to September 30, 1985

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:	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 reason this project was terminated is because the principle investigator has left.

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01571-03 SMRP

PERIOD COVERED

October 1, 1985 through September 30, 1985

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: M. Fujimoto, Visiting Associate, Laboratory of Preclinical Pharmacology

Others: H. Alho, Visiting Scientist, LPP; A. Guidotti, Section Chief, LPP; E. Costa, Lab Chief, LPP; I. Hanbauer, Pharmacologist

COOPERATING UNITS *(if any)*

Natl. Heart, Lung,Blood Inst., Div.Hypertension-Endocrine Branch, Bethesda, Md.

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Laboratory of PReclinical Pharmacology

SECTION

Neuroendocrinology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington DC 20032

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.)*

Adrenal chromaffin, cells from different mammalian species contain gamma-aminobutyric acid, glutamic acid decarboxylase, GABA aminotransferase, and GABA receptors. GABA and glutamic acid decarboxylase are also present in fibers impinging upon adrenal chromaffin cells. The membranes of chromaffin cells contain GABA/Benzodiazepine binding sites linked to Cl^- channels. Occupation of these receptors with GABA or GABA mimetics appears to modulate the acetyldoline-induced release of catecholamines and enkephalin-like peptides in parallel direction.

PHS 6040 (Rev. 1/84)

GPO 904-917

For several decades neuroscientists have been studying mammalian GABAergic transmission as if it were exclusive of brain and spinal cord. However, in the last few years growing evidence suggest the existence of a GABAergic system in peripheral nervous system which may be similar to that of brain.

To study whether GABA plays a physiological role in adrenal medulla we studied whether histochemical and biochemical markers of GABA are present in adrenal medulla and whether GABA receptor stimulation modulates the excretion of catecholamines and opiate peptides induced by stimulation of nicotine receptors.

We have shown that GAD, GABA, GABAT, the specific markers of GABAergic system, are present in fibers and in chromaffin cells of adrenal medulla of different mammalian species. Moreover, we have observed that cultures of bovine adrenal chromaffin cells can take up store and release ^{3}H-GABA. This release can be elicited by stimulation of nicotine receptors. The membranes of chromaffin cells contain GABA/benzodiazepine binding sites linked to Cl^- channels. Moreover, like the brain, in chromaffin cells, occupancy of GABA recognition sites by GABA elicited Cl^- channel opening.

In vivo studies with male foxhound dogs were performed to evaluate the functional role of a CABA in the adrenal medulla. Drugs were injected directly into the gland by the technique described by Hilton et al (Am. J. Physiol. 192-525-1958) and blood samples to measure CA and Met-^{5}Enkephalin-like immunoreactivity were obtained from the left adrenal vein.

THIP and other GABA receptor agonists increase the release of CA and Met-^{5}Enkephalin-like immunoreactive material in parallel. The release of CA and Met^{5}-Enkephalin-like immunoreactivity was not blocked by hexametonium maloxone or splanchnicotomy but was instead prevented by bicuculline.

These data suggest that GABA receptor stimulation release catecholamines and Met^{5}-Enkephalin-like immunoreactive material as a consequence of chromaffin cell depolarization.

We believe that GABA should be included in the list of important neuromodulators (Enkephalin substance P, somatostation, VIP) which control the responsiveness of chromaffin cells to incoming cholinergic stimuli and the adrenal medulla model appears of great importance to elucidate the physiological role of CABA in cholinergic transmission.

References:

Gamma-aminobutyric acid (GABA) in the adrenal medulla: Location, pharmacology and function. In: Neuropsychiatry Today, Alan Liss, 1985, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 MH 01572-03 SMRP

PERIOD COVERED

October 1, 1984 through September 30, 1985

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, LPP and M. R. Santi, LPP

Others: D. Konkel, Chemist, LPP; H. Alho, Visting Associate, LPP; A. Guidotti, Section Chief, LPP; E. Costa, Lab Chief, LPP

COOPERATING UNITS *(if any)*

B. Conti-Tronconi, Division of Chemistry and Chemical Engineering, California Inst. Technol., Pesadena, CA 91125

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Neuroendocrinology

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NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032 -

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	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.)*

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. 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 a human brain homogenized in hot (80^{0}) 1 N acetic acid. 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. Trypsin digestion of DBI within the DBI molecule an octadecaneuropeptide, ODN (1-18), which has biological activity stronger than that of DBI itself. These data suggest that DBI may function as a precursor od a putative endacoid of benzodiazepine recognition site.

Project Description:

DBI (diazepam binding inhibitor) extracted from rats or humans was purified to homogenity 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.

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. Using this sequence two independent laboratories (Dr. P. Seeburg, Genetech, Dr. J. Brosius and I. Mochetti, Columbia University, New York) have now isolated the cDNA clone for rat and human DBI.

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. Hypothalamus and cerebellum are 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 inner 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. However, coexistence of DBI within the GABAergic neurons is not a necessary event, and in fact high concentration of DBI is found in pyramidol, non GABAergic cells in Hippocampus and layer VI of cerebral cortex. The large molecular weight of DBI and its relatively weak potency (Ki in the μ M 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.

Trypsin digestion produces 7 major peptide fragments. Of these fragments only fragment T5 (MW of approximately 2000 daltons) has proconflict action. T5 was sequenced and shown to be an octadecaneuropeptide (ODN) with the following amino acid sequence: Gln-Ala-Thr-Val-Gly-Asp-Val-Asn-Thr-Asp-Arg-Pro-Gly-Leu-Arg-Leu-L^{18}ys. In vivo ODN 1-18, ODN 11-18, ODN 12-18 and ODN 13-18 all displace ^{3}H betacarbolines from their binding sites on

intact cerebellar granule cells. ODN was more potent (4 to 5 times) than DBI. Moreover ODN-1-18 α amide was ineffective in displacing ^{3}H Beta carbolines. ODN 1-18 but not ODN 1-18 α amide has proconflict action when injected intracerebroventricularly in rats. The proconflict action if ODN is more potent than that of DBI. Moreover using antibodies raised against ODN-1-18 it was found that ODN-like material is also present in rat brain. 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.

Publication:

Corda, M.G., Ferrari, M., Guidotti, A., Konkel, 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., 47:319-325.

Costa, E., Ferrari, M., Ferrero, P., and Guidotti, A.: Multiple signals in GABAergic transmission: Pharmacological consequences. Neuropharmacology, 23:989-991, 1985.

Ferrero, P., Guidotti, A., Conti-Tronconi, B., Costa, E.: A brain octadecaneuropeptide generated by trypic digestion of DBI (Diazepam Binding Inhibitor) functions as a proconflict ligand of beurodiazepine recognition sites. Neuropharmacology, 23:1359-1362, 1984.

Alho, H., Costa, E., Ferrero, P., Fujimoto, M., Cosenza-Murphy, D., Guidotti, A.: Diazepam binding inhibitor: A neuropeptide located in selected neuroed populations of rat brain. Science Vol. 229:170-182, 1985.

Costa, E., Guidotti, A.: Endogenous ligands for beurodiazepine recognition sites. Biochemical Phamacology, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01573-03 SMRP

PERIOD COVERED October 1, 1984 through September 30, 1985

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)*

P.I.: P. Ferrero, Guest Researcher, Laboratory of Preclinical Pharmacology

Others: A. Guidotti, Section Chief, LPP: E. Costa, Laboratory Chief, LPP

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Neuroendocrinology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, DC 20032

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.)*

Application of non-invasive brain imaging techniques to the study of neurotransmitter receptors make it necessary to develop animal models to detect in vivo modulation of receptor number and affinity. The modulation of Dopamine receptors in vivo was studied on rats injecting ^{3}H spiroperidol. In rats chronically treated with typical (haloperidol) and atypical (sulpiride) neuroleptics the number of ^{3}H spiroperidol binding sites was increased. The chronic neuroleptic induced increase in ^{3}H spiroperidol binding was selectively antagonized by the D_2 receptor antagonist sulpiride but not by the D_1 receptor antagonist SCH-23390. These data suggest that with the use of radiolabeled spiroperidol it is possible to study in vivo the modulation of the kinetics parameters of D_1 and D_2 Dopamine receptors. To study the GABA receptors we used ^{3}H muscimol injected in mice. Muscimol labels in a saturable manner and high affinity GABA receptor. Diazepan, 1mg/kg increases the Bmax of this binding. Since Diazepan potentiates the pharmacological responses of Muscimol these data suggest that the facilitatory action of Benzodiazepine on GABAergic transmission is mediated by an increase availability of GABA recognition site at the membrane site.

PHS 6040 (Rev. 1/84) GPO 904-917

Project Description:

In vitro measurement 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 ^{3}H-neuroleptic binding sites is increased. Moreover, binding sites in post mortem 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 post mortem binding studies to the in vivo situation, it is hoped that emission computed tomography (ECT) scanning, in its two modalites, that with gamma-emitters (single photo emission tomography SPECT) and that with positron emitters (PET) may be used to locate and diagnose DA receptor abnormalities in vivo in man. However 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.

For this purpose the kinetics of ^{3}H-spiroperidol binding to dopamine (DA) recogniton sites in different brain regions and a. pituitary of rat was studied ex vivo injecting intravenously ^{3}H-spiroperidol. Authentic spiroperidol ws separated from spiroperidol metabolites using reverse phase Sep Pak C-18 minicolumn. Pharmacological studies using drugs (SCH-23390 and sulpiride) that selectively bind to D_1 or D_2 DA receptor subtypes suggest that a tracer dose of ^{3}H-spiroperidol (Inmol/kg i.v.) selectively binds to D_2 recognition sites in a. pituitary ond to either D_2 or D_1 recognition sites in c. striatum. In the c. striatum and a. pituitary of rats receiving for 4 weeks two daily injections of haloperidol or l-sulpiride the number of ^{3}H-spiroperidol binding sites was increased. This chronic neuroleptic-induced increase of ^{3}H-spiroperidol binding was selectively antagonized by the D_2 receptor antagonist, sulpride, but not by the D_1 receptor antagonist, SCH-23390. The results suggets that with the use of radiolabeled position or gamma-emitting spiroperidol combined with other selective antagonist of D_1 or D_2 recognition sites subtypes, one could study animals and man DA D_1 or D_2 receptor modulation in vivo using emission computed tomography.

If it could be demonstrated that the ^{3}H-spiroperidol kinetic found in rat applies also to man, spiroperidol appropriately labeled could be used advantageously for ECT scanning. 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 vivo binding of ^{3}H-ligand to the GABA recognition sites.

Gamma-aminonutyric acid (GABA) receptors were characterized in vivo by studying ex vivo the binding of ^{3}H-muscimol to cerebellum, cortex, hippocampus, and corpus striatum of mice receiving intravenous injections of tracer doses of high specific-activity (30 Ci/mmol) ^{3}H-muscimol. This ligand binds with high affinity (apparent kd, 2-3 x 10^{-9}M) to a single population of binding sites (apparent Bmax, 250-280 fmol per 10 mg of protein).

Pharmalogical studies using drugs that selectively bind to GABA recognition site. Moreover, diazepam (1.5 mmol/kg, i.p.) increases the Bmax but fails to change the affinity of ^{3}H-muscimol binding to different brain areas. This diazepam-elicited increase in Bmax is blocked in mice receiving the diazepam agonist, RO 15-1788 (ethyl-8-flouro-5,6-dihydro-5-methyl-6-oxo-4H-imidazo (1,5a)(1,4) benzodiazepine-3-carboxylate). Since the diazepam induced increase of ^{3}H-muscimol binding is parallel to a significiant 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 increse in the Bmax of the binding sites of $GABA_A$ receptors. We intended to continue the characterization of D_1 and D_2 receptors 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:

Guidotti, A. and Ferrero, P.: Ex vivo binding of ^{3}H-muscimol to GABA recognition sites: A tool to charaterize GABA receptor agonists - In Bartholini, G (ed): Epilepsy and GABA Agonists: Basic and Therapeutic Research, NY, Raven Press, pp. 31-49, 1985.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01574-03 SMRP

PERIOD COVERED

October 1, 1984 to September 30, 1985

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)*

P.I.	H. Kageyama	Guest Reserchmer	LPP-NIMH
Other:	A. Guidotti	Section Chief	LPP-NIMH

COOPERATING UNITS *(if any)*

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LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Neuroendocrinology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	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 goal of this project was to develop a radiolabelled ligand for nicotinic receptors by iodinating a polypeptide extracted and purified in our laboratory from the Bungarus multicintus venom. This peptide had the ability to inhibit nicotinic receptor function in adrenal medulla and sympathetic ganglion. However labeling of this peptide deteriorated its biological activity and therefore the project had to be terminated.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 MH 01577-02 SMRP

PERIOD COVERED

October 1, 1984 to September 30, 1985

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:	Chuang	Chemist
Other:	Ora Dillon Carter	
	T. Nakaki	(Visiting Fellow)
	E. Costa	Lab Chief

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:
1.6	1.6	none

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.)*

NCB-20, a cloned hybrid cell line of mouse neuroblastoma and fetal Chinese hamster brain cell, has been used as a model system for the study of receptor-receptor interactions in the same cell. NCB-20 cells have 5-HT receptors which are linked to adenylate cyclase. The plasma membranes of these cells also contain high affinity binding sites for 3H-ketanserin and 3H-mianserin. In addition, these cells are equipped with 5-HT presynaptic components. These include a 5-HT uptake system and a very high Bmax of 3H-imipramine binding sites (50-times the Bmax in the CNS). 5-HT can release acetylcholine from NCB-20 cells. When phosphoinositides in the cells were prelabeled by preincubation with 3H-inositol, the addition of carbachol enhances the accumulation of 3H-inositol monophosphate (in the presence of Li^+) by about 400%. Significant increase of ^{3}H-inousitol bisphosphate formation can also be detected. This carbachol-induced activation of phospholipase C is time dependent, dose-related (EC50 10^{-5}M) and blocked by atropine. These data provides the first evidence for the presence of functional muscarinic acetylcholine receptors in NCB-20 cells and suggests that acetylcholine released by 5-HT may autoregulate the hydrolysis of phosphoinositides. The information obtained in this model system may gain insight of the molecular mechanisms of receptor-receptor communications in the same neuron.

PHS 6040 (Rev. 1/84)

GPO 904-917

Project Description:

The understanding of the molecular mechanisms of the interactions between receptors for neurotransmitters in CNS has been hampered by the complexity of brain structures. Part of this complexity arises from the presence of heterogenous cell populations which include not only neurons but also glial cells. This understanding may be facilitated by the use of a model system of a cloned cell line which contains multiple receptors for neurotransmitters. We have found that NCB-20, a cloned hybrid cell line of mouse neuroblastoma and fetal Chinese hamster brain cell could be such a model system.

In confirming the observations by Nirenberg and coworkers (MacDermot et al., PNAS 76: 1135-1139, 1979), we have found that NCB-20 cells have 5-HT sensitive adenylate cyclase which can be blocked by ketanserin, a selective 5-HT_2 receptorant agonist. We have also found that the plasma membranes of these cells contain specific ^{3}H-ketanserin binding sites (Kd = 7.4±2.8 nM; Bmax = 0.63±0.11 pmol/mg prot). Specific binding for ^{3}H-mianserin can also be detected (Kd = 10.8±2.3 nM; Bmax = 1.51±0.56 pmol/mg prot). Our previous studies in rat brain have shown that mianserin, an atypical antidepressant is bound to a site closely related but not identical to the 5-HT_2 receptor site (labeled by ^{3}H-ketanserin at the serotonin synapses). Interesting enough, NCB-20 cells are also equipped with serotonin presynaptic components. These include a 5-HT uptake system and a high affinity binding site for imipramine, a classical tricyclic antidepressant drug. Studies by us and others in the CNS have suggested that imipramine is bound to a presynaptic site controlling the uptake of 5-HT in a negative manner. It is of importance to mention that the density of imipramine binding site in NCB-20 cells (16±6 pmol/mg prot) is at least 50 times higher than that in the brain. Thus NCB-20 cell line is a valuable source for imipramine binding site isolation and characterization. Moreover, since serotonin synapse has been implicated to be an important site of action of antidepressant drugs, NCB-20 cells can also be used to study the molecular events following the binding of these drugs to their "receptor" sites such as interactions of these drug receptors with receptors for neurotransmitters. These studies are now in progress.

It has also been shown by MacDermot et al., that 5-HT can release acetylcholine from NCB-20 cells; however, the 5-HT receptor responsible for acetylcholine release is distinct from the 5-HT receptor mediating the activation of adenylate cyclase. Recent data indicate that muscarinic, acetylcholine (M1) receptor in brain slices and cultured tumor gliomas is linked to phospholipase C which hydrolyzes phosphoinositides. We have therefore examined the possibility of whether acetylcholine released by 5-HT in NCB-20 cells can activate phospholipase C. When NCB-20 cells are preincubated with ^{3}H-inositol to label inositol-containing phospholipids in the presence of LiCl (which blocks the inositol-monophosphate specific phosphatase), we found that the addition of carbachol increases the accumulation of inositol-monophosphate by about 400%. This carbachol-induced activation of phospholipase C is time dependent (linear up to 30 min), dose-related (EC_{50} 10^{-5}M) and blocked by atropine (1 uM). The formation of ^{3}H-inositol bisphosphate is also increased significantly by carbachol but the onset of the increase is faster than that of ^{3}H-inositol monophosphate. These data has provided the first evidence for the presence of functional muscarinic receptors in NCB-20 cells. We are now investigating whether 5-HT can activate phospholipase C in this cell line and, if so, whether this activation is due to a direct stimulation of 5-HT receptor or is mediated by the release of acetylcholine which in turn stimulates the M1 acetylcholine receptor. We are also studying whether the activation of phospholipase C by carbachol or other transmitters triggers mobilization of intracellular Ca^{2+} and stimulation of protein kinase C. The physiological protein substrates for protein kinase C in NCB-20 cells is also being investigated.

Because NCB-20 cells are equipped with unusually high numbers of different types of neurotransmitter receptors and drug binding sites, this cell line is ideal for the study of multiple receptor interactions at the functional and biochemical levels. The information obtained from this model system may lead to a better understanding of the communication between receptors in the same cell and eventually provide a new basis for the treatment for some mental illnesses which are related to abnormalities of receptor-receptor interactions.

Publication:

Nakaki, T., Roth, B.L., Chuang, D.M., and Costa, E.: 5-HT uptake and imipramine binding sites in neurotumor NCB-20 cells. J. Neurochem., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 MH 01578-02 SMRP

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Expression of genes for insulin in brain and peripheral tissues

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

PI: D.M. Chuang

Others: T.T. Quach

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:
1.2	1.2	none

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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 study addresses the question of whether mRNAs for insulin and/or insulin-like peptides are present in the brain and other extrapancreatic tissues in an attempt to understand the role of these peptides in the maintenance of mental health. RNAs are extracted from various tissues using liquid nitrogen pulverization followed by homogenization in the presence of GuSCN. The RNA pellets recovered from CsCl-cushion centrifugation of the homogenate are subjected to oligo-dt columns for the purification of poly A^+ RNAs which include mRNAs. These isolated mRNAs are electrophoresed on agarose gel followed by blotting to a nitrocellulose membrane. These immobilized mRNAs are then hybridized to a cloned cDNA fragment of proinsulin gene which has been nick-translated with ^{32}P-dCTP. We found that the ^{32}P-cDNA probe is hybridized to mRNA from extrapancreatic tissues under stringent conditions. However, the molecular sizes of these hybridizable mRNA transcripts are different from that detected in the pancreas. Thus, the size of pancreatic mRNA is of 0.5 kilobase, whereas two species of mRNA transcripts detected in the gut, heart and to a lesser extent, liver have approximately 4.2 and 2.2 kilobases. We also detected these two mRNA transcripts in the brain and a cloned cell line NCB-20 (neuroblastoma x fetal hamster brain cell hybrid), suggesting a neuronal location of these transcripts. The nature and function of the proteins translated from these extrapancreatic mRNA is now under investigation.

Project Description

The insulin genes are part of an evolutionary related gene family, since proinsulin is similar in structure and amino acid sequence to at least two other insulin like growth factors (IGF-I and IGF II). This homology has been defined for IGF-I at both the amino acid and nucleotide levels and IGF-II at the amino acid level (Rinderknecht, E. and Humbel, R., J. Biol. Chem. 253: 2769-2776, 1978). It has been reported that both IGF-II (Haselbacher et al., PNAS 82: 2153-2157, 1985) and substances reactive to insulin antibody (Havrankova et al., Nature 272: 827, 1978) are present in the brain. However, neither the nature nor the origin for these polypeptides has been clearly defined. In fact, because of the low quantity of insulin (defined by reactivity with an insulin antibody), it has been suggested that brain insulin is blood borne. The present study was designed to assess this question using a cloned cDNA fragment of proinsulin (kindly provided by Dr. Villka-Komaroff, Univ. of Massachusetts) to examine for the presence of mRNAs in the rat brain and other extrapancreatic tissues that can be hybridized to this cDNA probe.

RNAs are extracted from various tissues of adult rats using liquid nitrogen pulverization followed by homogenization in the presence of GuSCN. The RNA pellets resulting from CsCl-cushion centrifugation of the tissue homogenates are subjected to oligo-dt columns for the purification of poly A^+ RNAs which include mRNAs. These isolated mRNAs are further electrophoresed on a agarose gel followed by blotting to a nitrocellulose membrane. These immunobilized mRNAs are then hybridized to the proinsulin cDNA which has been nick-translated with ^{32}P-dCTP. We found that the ^{32}P-cDNA probe is hybridized to mRNAs from several extrapancretic tissues under stringent conditions (42^{o}C with 0.8 N NaCl and 50% formamide). However, the molecular sizes of these hybridizable transcripts are different from that detected in the pancreas. Thus, the size of pancreatic hybridizable mRNA is approximately 0.5 kilobase, whereas two species of RNA transcripts hybridized to probe are detected in the gut, heart, and to a lesser extent, the liver. A rough estimation of their sizes using ribosomal RNAs as the marker reveals that they have 4.2 and 2.2 kilobases. Interestingly, we also found that there are two species of hybridizable RNA transcripts in the brain with approximately the same size. Moreover, these two RNA transcripts are also detected in cultured cells of NCB-20 which is a cloned line of mouse neuroblastoma x fetal chinese hamster brain cell. These results suggest that these transcripts in the brain are of neuronal origin. Since insulin (or insulin-like peptide) is detectable in the brain, one may infer that these brain RNA transcripts are translated into proteins. However, the nature of these proteins is currently unknown. It should be stressed that the level of these RNA transcripts in extrapancreatic tissues is at least 50 times lower than that of proinsulin mRNA in the pancreas. The low abundance may explain the failure of previous investigators to detect their presence in these expancreatic tissues of adult rats.

It has been shown previously by us that the addition of insulin to olfactory bulb slices of rat modulates the production of cAMP that is increased by dopamine (Barbaccia et al., Regulatory Peptides: From Molecular Biology to Function, pp 511-518, 1982). It is possible that insulin or insulin-like peptides encoded by the mRNA transcript detected in the present study may function as a neuromodulator. In light of the growth promotion activity endowed with IGF-I in some systems it is also likely that these putative brain insulin-like peptides are nerve tropic factors required for the naturation of neurons. Oncogenic studies and regional distribution of these brain mRNA are now in progress. This study should lead to a better understanding of the molecular nature of insulin and/or insulin-like peptides in the CNS and other pancreatic tissues and their role in the maintenance of functional equilibrium of our mental states and other physiological activities.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 01579-02 SMRP

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Studies of an endacoid for the $5HT_2$ recognition site

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

P.I.	B.L. Roth	Guest Researcher	LPP-NIMH
Other:	D.M. Chuang	Chemist	LPP NIMH
	T. Nakaki	Visiting Fellow	LPP NIMH
	E. Costa	Lab. Chief	LPP 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:
1.8	1.8	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.)*

5-HT_2 receptor binding sites are not up-regulated following denervation of serotonin terminals by a selective neurotoxin, suggesting that endgenous substance other than 5-HT exist for this receptor sites. We have, therefore, attempted to identify and isolate endogenous ligands for the 5-HT_2 recognition site in the CNS. When 0.1 M acetic acid extract of bovine brain is applied a CM-Sephadex column, some fractions display activity of inhibiting ^{3}H-ketanserin binding to the rat brain membranes. This activity is diminished by treatment with trypsin or pronase, suggesting that the material is protein in nature. This polypeptide has a molecular weight of about 6,000 daltons and inhibits the specific binding of 3H-Ketanserin and, to a slightly lesser extent ^{3}H-mianserin, but fails to affect the binding of ^{3}H-imipramine and ^{3}H--dihydroalprenolol. This peptide has been partially purified by carboxylmethyl-sephardex chromatography, Biogel P-10 permeation chromatography and a reversed phase HPLC C_8 column chromatography. We have also tested alternative extraction procedures using 0.1 M HCl and various concentractions of organic solvent (such as accetonitrile) in an effort to maximize the recovery and to facilitate the purifiction to homogeneity of this protein. We are currently testing the biological activity of this polypeptde on 5-HT_2 receptors by ascertaining its effects on (1) 5-HT sensitive adenylate cyclase activity in NCB-20 cells, (2) 5-HT stimulated phosphoinositide hydrolysis in brain slices and aorta of rats and (3) 5-HT-induced contraction of the ring of rat aorta. Since 5-HT_2 receptor recognition sites are down-regulated following subchronic treatment of experimental animals with a variety of antidepressnats, this putative peptide may be involved in this affective disorder and might be used as a biological marker for this mental illness.

PHS 6040 (Rev. 1/84) GPO 904-917

Project Description:

Serotonin has been implicated to be an important site of action of antidepressant drugs. Evidence has accummulated, suggesting that there might exist at least two classes of serotonin (5-HT) recognition sites in mammalian brain. These sites have been designated 5-HT_1 and 5-HT_2 and show differential pharmacologic specificity. In brief, the 5-HT_1 site binds ^{3}H-5HT with high affinity (kd in nM ranges) is regulated by guanine nucleotides and may be coupled to adenylate cyclase. The 5-HT_2 site binds 5-HT with somewhat lower affinity (Kd 20 uM) is not regulated by guanine nucleotides and binds certain 5-HT antagonists (e.g., ketanserin and mianserin) with very high affinity (Kd in nM ranges). In addition, the 5-HT_2 recognition site appears to mediate certain behavioral effects and peripheral vascular contraction caused by 5-HT. We have recently shown that 5-HT_2 receptor recognition sites in rat aorta are linked to by phosphoinositide hydrolysis by phospholipase C. Using ^{3}H-mianserin and ^{3}H-ketanserin as ligands. We have previously shown that these two compounds might be labeling distinct recognition sites. Since pharmacological manipulations which would be expected to result in supersensitivity of 5-HT_2 recognition sites (e.g. lesioning of 5HT nerve terminals) affected only the 3H-mianserin recognition site, it was suggested that 5-HT might not have been the major endogenous ligand for the 5-HT_2 recognition site. We have therefore initiated a search for an endocoid for the 5-HT_2 recognition site.

In our routine protocol, 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 the inhibition of ^{3}H-ketanserin binding to rat brain membranes 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 column and eluted with 70% acetonitrile; the resulting material is applied to a Bio Gel P-10 column. One major active fraction (MW approx. 6000 daltons) and a few minor active fractions are collected and then applied to a reversed phase HPLC C-8 column, because it has been suggested that this HPLC column is most effective in purifying polypeptides with relatively small molecular weights.

We have found that with the most active fractions as little as 1-3 micrograms of partially purified peptide inhibits at least 50% of ^{3}H-ketanserin specific binding. To a slightly lesser extent and with a weaker potency, this isolated peptide also inhibited the specific binding of ^{3}H-mianserin to rat brain membranes. However, there was little specific inhibition of B-adrenergic, imipramine or 5-HT_1 binding to rat cortical membranes. We have also assessed the protease sensitivity of the partially purified peptide and found that 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-HT_2 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 also found that the peptide inhibits ^{3}H-ketanserin binding to the plasma membranes of NCB-20 cells, suggesting that it interacts with the 5-HT_2 recognition sites on these cells as well as in the brain. We are also examining the effect of this endogenous peptide on the hydrolysis of phosphoinositides in brain and aorta of rats. In this study, brain slices or intact aorta will be preincubated with ^{3}H-inositol to label phosphoinositides and the 5-HT-induced hydrolysis of these inosibol-containing phospholipids will be examined in the presence or absence of this endocoid. We have recently shown that various 5-HT_2 receptor antagonists potently inhibited the 5-HT stimulated phosphoinostitide hydrolysis in rat aorta, suggesting that aortic 5-HT_2 recognition sites are linked to phospholipase C. Furthermore, we have noted strong correlation between phosphoinositide hydrolysis and aortic contraction. Finally, we found that biologically active phorbol esters inhibited 5-HT stimulated phosphoinositide hydrolysis (Roth et al, in preparation).

We are also attempting to optimize the isolation conditions in an effort to maximize the recovery of this peptide. Alternative conditions being examined include extractions with 0.1 M HCl and various concentrations of organic solvents such as acetonitrile. Our ultimate goal is to purify this protein to homogeneity and to elucidate the structure of this protein endocoid. Since 5-HT_2 receptors are known to be down-regulated by repeated treatments with antidepressant drugs in experimental animals, this putative endocoid may be involved in the affective disorders. Characterization of this protein endocoid in the cerebral spinal fluid of patients with affective disorders may help elucidate the etiology of this mental illness.

Publication:

Roth, B.L., Nakaki, T., Chuang, D.M. and Costa, E.: Evidence for an endocoid for the 5-HT_2 recognition sites. In Lal, H. (Ed): Proceedings of the First International Endocoid Symposium. New York, A.P. Liss, Inc. in press

Roth, B.L. Nakaki, T., Chuang, D.M. and Costa, E.: Aortic recognition sites for 5-HT are coupled to phospholipase C and modulate phosphatidylinositol turnover. Neuropharmacology, 23, 1223-1225. 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: ZO1 MH 01580-01 SMRP

PERIOD COVERED
October 1, 1984 through September 30, 1985

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

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:
0.9	0.9	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.)*

The reason this project was terminated is because the principle investigator has left.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 01583-02 SMRP

PERIOD COVERED: October 1, 1984 to September 30, 1985

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)*

P.I.	A. Novelli	Guest Researcher	LPP-NIMH
OTHER:	A. Guidotti	Section Chief	LPP-NIMH
	F. Nicoletti	Guest Researcher	LPP-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:	PROFESSIONAL:	OTHER:
0.1	0.1	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.)*

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. This increse in cGMP content following the application of different excitatory amino acids to the cultures has been found to be dependent on the ionic component of the medium and Mg^{++} in particular. This observation will be a departure point to study the interactions among kainic acid, aspartate, and glutamate recognition sites at the cellular level, and to characterize whether kainic acid receptors have a physiological role or participate only in neurodegenerative disorders.

PHS 6040 (Rev. 1/84) GPO 904-917

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 GMP response to various excitatory dicarboxylic amino acids in 8 day old primary cultures of rat cerebellar granule cells.

We found that in Locke's solution 10^{-4}M KA produces up to a 100-fold increase in cGMP content which is the maximum after 1 min (ED_{50} $5x10^{-5}$M). This increase is not due to an inhibition of the phosphodiesterase activity by KA since maximally inhibitory concentrations ($5x10^{-4}$M) of 3-isobutyl-1-methylxantine, an inhibitor of the phosphodiesterases is able to potentiate the cGMP increased 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. A 2- to 5-fold increase of cGMP is also produced by glutamic acid and aspartic acid. In contrast, N-methyl aspartic (NMDA) up to 10^{-3}M failed to increase cellular cGMP content. However, when the glutamic aspartic acid and NMDA are applied in Mg^{++} free Locke's solution they are equipotent with KA and produce at 10^{-4}M concentrations up to a 100-fold increase in the cGMP content. The increase of cGMP induced by KA is antagonized by 2,3-cis-piperidindicarboxylic acid (1PDA) preferentially, while the increased induced by glutamic aspartic acid and NMDA is antaagonizedby Dl-2-amino-5-phosphonovaleric acid (APV) preferentially.

Moreover, 10^{-6}M bicuculline, a $GABA_A$ receptor blocker, fails to alter the KA induced increase of cGMPcontent. These data suggest the existence of a selective relationship between the dicarboxylic excitatory amino acid receptors and cGMP. Since guanylate cyclase is a soluble enzyme the understanding of the molecular mechanisms of this interconnection between excitatory amino acid receptors and guanylate cyclase is not easy. Probably an interposed 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 excitatory amino acid and the molecular mechanism involved in the activation of guanylate cyclase.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01584-02 SMRP

PERIOD COVERED

October 1, 1984 to September 30, 1985

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)*

PI:	R.B. Rothman	Guest Researcher	DIRP	NIMH
Others:	M. Herkenham	Staff Scientist	LNP	NIMH
	A.E. Jacobson	Staff Scientist	LC	NIADDKD
	K.C. Rice	Staff Scientist	LC	NIADDKD
	J. Danks	Guest Researcher	DIRP	NIMH
	S. McLean	Staff Fellow	LNP	NIMH

COOPERATING UNITS *(if any)*

Walter Reed Inst. Res., Washington, D.C.; Lab. Neurophysiol., NIMH; Lab. Chemistry, Natl. Inst. Arthritis, Diabetes, Digestive and Kidney Diseases, Bethesda, Md.

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:	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.)*

A major focus of work has been to study the use of site-directed alkylating agents to define opiate receptor subtypes. Previous work demonstrated that pretreatment of membranes with the fentanyl derivative FIT eliminates the higher affinity 3H-D-ala2-D-leu5-enkephalin binding site. Additional work has shown that this delta-selective irreversible ligand in addition alters the properties of the lower affinity 3H-D-ala2-D-leu5-enkephalin (3H-DADL) binding site. Additional experiments have shown that pretreatment of membranes with both FIT and the mu-receptor directed irreversible ligand, BIT, produces a membrane preparation highly enriched with kappa opiate receptors. Using this protocol, the distribution of kappa receptors in the hippocampus of several species were determined using autoradiographic techniques. Of some interest is the finding that opiate receptors in the rat pituitary are confined to the neural lobe and are exclusively kappa receptors. A second line of investigation has been to identify in vivo manipulations of rats that will produce selective alterations in opiate receptors assayed in vitro. Thus using the site-directed alkylating agents to define 3H-DADL binding sites, we have shown that whereas chronic morphine causes an up regulation of the lower affinity 3H-DADL binding site, chronic electroconvulsive shock causes a down-regulation of this binding site. It is likely that these observations may lead to a greater understanding of the mechanisms underlying the development of tolerance and dependence to opiates.

Project Description:

The primary goal of this project is to document and explore noncompetitive interactions between opiate receptors. The overall working hypothesis is that there exists an opiate receptor complex which possesses mu, delta, and kappa binding sites, and in addition "pure" mu, delta, and kappa receptors not associated with the receptor complex. Thus previous work has shown that the enkephalin analog ^{3}H-D-ala^{2}-D-leu^{5}-enkephalin (^{3}H-DADL) labels two binding sites distinguished by the inhibitory mechanism of mu ligands. Thus whereas mu ligands are competitive inhibitors at the higher affinity ^{3}H-DADL binding site (mu-competitive binding site), they are noncompetitive inhibitors at the lower affinity binding site (mu-noncompetitive binding site). Based upon the finding of noncompetitive interactions, we concluded that the mu-noncompetitive binding site was the delta binding site of the receptor complex and that the noncompetitive interaction was mediated via an adjacent mu binding site.

Using similar approaches, we have begun to explore the kappa arm of the working hypothesis. Using ^{3}H-bremazocine to label brain membranes, we have established assay conditions in which this ligand labels only kappa binding sites. This has been accomplished using site directed alkylating agents which eliminate mu and delta binding sites. Preliminary studies indicate that delta ligands are competitive inhibitors at one class of kappa sites and noncompetitive inhibitors at a second class of kappa sites.

Work continues on the mu arm of the model. Previous work indicated that ^{3}H-naloxone labeled a mu site at which delta ligands are noncompetitive inhibitors, but no compelling evidence for a mu site not associated with the complex came out of this study. We are continuing this line of investigation using the novel antagonist ^{3}H-cyclo-FOXY, which is a PET scanning ligand, and the alkylating agent beta-FNA. ^{3}H-cyclo-FOXY gives a superior signal-noise ratio which makes possible the use of more favorable assay conditions. This work is still in progress, and the results are not available at this time.

A simple question set in motion the next set of investigations. Is the mu-noncompetitive ^{3}H-DADl binding site on the same molecule as the mu site labeled by ^{3}H-naloxone. To answer this question we hoped to find an in vivo manipulation which would selectively up- or down-regulate the mu-noncompetitive binding site. If ^{3}H-naloxone labeled the same macromolecular complex, then its binding site should be similarly affected. We have now shown that chronic morphine causes a selective up-regulation in the mu-noncompetitive binding site, and that chronic electroconvulsive shock causes a selective down-regulation. Using these two in vivo manipulations, we hope to explore the relationship between binding sites defined by mechanistic phenomena observed in vitro.

Finally, out of this work naturally falls out well-defined conditions with which to assay subtypes of opiate receptors. These conditions have been utilized in collaboration with members of the LNP to visualize using autoradiographic techniques the anatomical distributions of these subtypes of opiate receptors.

The proposed course of this project is to:

1. Continue to explore the kappa- and mu-arms of the working hypothesis using site-directed alkylating agents.
2. Use the chronic morphine and chronic ECS models as a tool to explore the relationships between binding sites defined by work in section 1.

3. In that the alteration in an opiate receptor subtype which occurs in addicted rats appears to be a relevant biochemical marker for addiction, we will utilize this marker to study the mechanism of tolerance and dependence in the CNS.
4. Explore the interaction between the endogenous opiate antagonists isolated and purified by Dr. Yang and associates in the LPP and the opiatergic systems using ligand binding techniques.

Significance of this study for biochemical research:

1. The opiatergic system is a model for the other peptidergic systems in the CNS. Advances in the understanding of this peptide system will blaze the trail for the many other neurotransmitter systems which utilize peptides.
2. Further definition of opiate receptor subtypes may lead to the development of more selective therapeutic agents.
3. A more thorough understanding of tolerance and dependence at the molecular level may lead to more effective treatments for individuals addicted to opiate drugs.

Publications:

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. 309: 47-54, 1984.

Rothman, R.B., Schumacher, U.K., and Pert, C.B.: Effect of beta-FNA on opiate delta receptor binding. J. Neurochem. 43: 1197-1200, 1984.

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Rothman, R.B., Pert, C.B., Jacobson, A.E., Burke, T.R., and Rice, K.C.: Morphine noncompetitively inhibits the binding of ^{3}H-leucine enkephalin to a preparation of rat brain membranes lacking type II-delta receptors. Neuropeptides 4: 257-260, 1984.

Rothman, R.B., Danks, J.A., Pert, C.B., Jacobson, A.E., Burke, T.R., Jr., and Rice, K.C.: Ionic condition differentially affect ^{3}H-DADL binding to type-I and type-II opiate delta receptors in vitro. Neuropeptides 4: 261-268, 1984.

Rothman, R.B., Daski, J.A., Jacobson, A.E., Burke, T.R., Jr., Rice, K.C., and Pert, C.B.: Tritiated-6-beta-fluoro-6-desoxy-oxymorphone: A highly selective ligand for the opiate mu receptor whose binding is characterized by low nonspecific binding. Neuropeptides 4: 311-317, 1984.

Rothman, R.B., Danks, J.A., Herkenham, M., Cascieri, M.A., Liang, T., and Pert, C.B.: Autoradiographic localization of a novel peptide binding site in rat brain using the substance P analog, eledoisin. Neuropeptides 4: 343-349, 1984.

Rothman, R.B., Bowen, W.D., Herkenham, M., Jacobson, A.E., Rice, K.C. and Pert, C.B.: A quantitative study of ^{3}H-D-ala^{2}-D-leu^{5}-enkephalin binding to rat brain membranes: Evidence that oxymorphine is a noncompetitive inhibitor of the lower affinity delta binding site. Mol. Pharmacol. 27: 399-409, 1985.

Rothman, R.B., Danks, J.A., Herkenham, M., Jacobson, A.E., Burke, T.R., and Rice, K.C.: Evidence that the delta-selective alkylating agent, FIT, alters the mu-noncompetitive opiate delta binding site. Neuropeptides, in press.

Rothman, R.B., Danks, J.A., Jacobson, A.E., Burke, T.R., Jr., and Rice, K.C.: Leucine enkephalin noncompetitively inhibits the binding of ^{3}H-naloxone to the opiate mu-recognition site: Evidence for delta---mu binding site interactions in vitro. Neuropeptides, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-MH 01585-01 SMRP

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Mechanisms of Smooth Muscle Cell Contraction in Rat Aorta

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	Chief	SMRP	NIMH

COOPERATING UNITS *(if any)*
Naval Medical Research Institute, Surgical Res. Br., Bethesda, Md.

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:
1.8	1.8	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 smooth muscle of rat aorta was used as a model system for the study of the molecular mechanisms of 5-HT2 receptor function. The 5-HT2 receptor-mediated contraction of rat aorta could be dissected into two distinct components (phasic and tonic) by the use of appropriate inhibitors; nifedipine, an inhibitor of voltage-dependent Ca2+ channels, inhibited only the phasic component of 5-HT-induced contraction while totally blocking the KCl-induced contraction. 2-Nitro-4-carboxyphenyl-N,N-diphenylcarbamate (NCDC), an inhibitor of phospholipase C, inhibited the tonic components of 5-HT-induced contraction. This component of contraction was mimicked by a protein kinase C activator, 12-O-tetradecanoylphorbol-13-acetate. The activity of phospholipase C was activated by 5-HT (500% increase) with an EC50 of about 10 μM which was nearly identical to the dose response noted for 5-HT-induced contraction. The potency order of the antagonists was consistent with the view that 5-HT-induced activation of phospholipase C is mediated through 5-HT2 receptors. The phospholipase C inhibitor NCDC inhibited the 5-HT induced increase in phospholipase C activity in rat aorta. Phosphorylation in a cell-free system of rat aorta showed that addition of calcium and phosphatidylserine increased the phosphorylation of some aortic proteins. These protein phosphorylations were also increased by protein kinase C activators such as 12-O-tetradecanoylphorbol-13-acetate (TPA) and 1,2-diolein implying that these proteins are the physiological substrates for protein kinase C in rat aorta. This study suggests that 5-HT2 receptors differentially regulate a voltage-dependent Ca2+ channel and phospholipase C activity. This study may provide a model system for the study of the signal transduction of the brain 5-HT2 receptor.

PHS 6040 (Rev. 1/84) GPO 904-917

Project Description:

Recent evidence suggests that extracellular informational signals in a variety of systems are transduced into the cellular interior through activation of phospholipase C which hydrolyzes phosphoinositide to form inositol trisphosphate and diacylglycerol; these two products then act synergistically on protein kinase C to evoke subsequent physiological responses. In smooth muscle, epinephrine and 5-hydroxytryptamine (5-HT) are known to cause muscle contraction through activation of α_1 and 5-HT_2 receptors respectively. We have attempted to use rat aorta as a model system to study whether the activation of phospholipase C and protein kinase C is involved in the aortic contraction induced by neurotransmitters.

We have found that the 5-HT-induced contraction could be dissected into two distinct components (phasic and tonic) by the use of appropriate inhibitors; nifedipine, an inhibitor of voltage-dependent Ca^{2+} channels, inhibited only the phasic component of 5-HT-induced contraction while totally blocking the KCl-induced contraction. 2-Nitro-4-carboxyphenyl-N,N-diphenylcarbamate (NCDC), an inhibitor of phospholipase C, inhibited the tonic components of 5-HT-induced contraction. This component of contraction was mimicked by a protein kinase C activator 12-O-tetradecanoylphorbol-13-acetate (TPA) in the presence of a Ca^{2+} ionophore A23187.

In collaboration with Dr. B. Roth at Naval Medical Research Institute, we have assayed the activity of phospholipase C in rat aorta rings by measuring the accumulation of ^{3}H-inositol monophosphate in the presence of lithium which blocks the conversion of inositol monophosphate to free inositol. The accumulation of ^{3}H-inositol monophosphate was activated by 5-HT (500% increase) with an EC50 of about 10 μM which was nearly identical to the dose response required for 5-HT-induced contraction. Specific 5-HT_2 antagonists inhibited the 5-HT induced activation of ^{3}H-inositol monophosphate accumulation with high affinity. Ketanserin, metergoline, pizotifen and mianserin had IC50's of between 3 and 10 nM, whereas amitryptline, and haloperidol had IC50's of about 100 nM. Prazosin, yohimbine and atropine had IC50's much greater than 1000 nM. The potencies of these antagonists were highly corelated with their abilities to inhibit ^{3}H-ketanserin binding but not with those to inhibit either 5-HT stimulated adenylate cyclase or ^{3}H-5-HT binding in the rat brain. Finally, the accumulation of ^{3}H-inositol monophosphate in rat aorta was inhibited by the phospholipase C inhibitor NCDC.

Our data of the aortic contraction suggested an involvement of protein kinase C in the 5-HT-elicited contraction. To examine this hypothesis, we carried out a phosphorylation study in a cell-free system to examine whether there are substrate proteins for protein kinase C in rat aorta. Ca^{2+} caused an increase in phosphorylation of several proteins. These include 16-, 20-, 36-, 45-, 48-, 60-, 92.5-kilodalton proteins. Among these proteins, an addition of phosphatidylserine to the Ca^{2+}-containing mixture further increased the phosphorylation of proteins of 16, 20 and 92.5 kilodaltons. We have also examined the effects of TPA and 1,2-diolein which are activators of protein kinase C and the biologically inactive analogue 4 -phorbol-12,13-didecanoate (4 -PDD). TPA and 1,2-diolein, but not 4 -PDD, increased protein phosphorylation of molecular weight 20, 88 and 92.5 kilodaltons. Therefore, in rat aorta we might have identified substrate proteins (20 and 92.5 daltons) for protein kinase C, which could be activated by 5-HT_2 receptor stimulation. Our future study is to prepare primary culture of smooth muscle cells of rat aorta to confirm the physiological substrate proteins for protien kinase C following 5-HT_2 receptor activation and to correlate this protein phosphorylation with the contraction of smooth muscle cells induced by 5-HT.

In summary, our results suggest that 5-HT_2 receptors differentially regulate a voltage-dependent Ca^{2+} channel and phospholipase C activity; the voltage-dependent Ca^{2+} channel is involved in the phasic component of contraction whereas the phosphoinositide hydrolysis that results in the activation of protein kinase C by diacyl glycerol and calcium mobilization by inositol trisphosphate plays a physiologically important role in the tonic component of the aortic contraction. These results also predict the phospholipase C or protein kinase C inhibitors might be potent vasodilators of great potential for use in critically ill patients.

Publications:

Roth, B.L., Nakaki, T., Chuang, D.M., and Costa, E.: Aortic recognition sites for serotonin are coupled to phospholipase C and modulate phosphatidylinositol turnover. Neuropharmacology 23: 1223-1225, 1984.

Roth, B.L., Nakaki, T., Chuang, D.M., and Costa, E.: Characterization of 5-HT_2 receptor linked to phospholipase C in rat aorta. Fed. Proc. 69: 179, 1985.

Nakaki, T., Roth, B.L., Chuang, D.M., and Costa, E.: Phasic and tonic components in 5-HT_2 receptor-mediated rat aorta contraction: Participation of Ca^{2+} channels and phospholipase C. J. Pharmacol. Exp. Ther., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-MH 01586-01 SMRP

PERIOD COVERED

October 1, 1984 through September 30, 1985

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

Immunohistochemical Studies on DBI and GABA in the Nervous System

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

Hannu Alho, Visiting Associate, Laboratory of Preclinical Pharmacology

Alessandro Guidotti, M.D., Medical Officer, Laboratory of Preclinical Pharmacology; Dr. Erminio Costa, M.D. Chief, Laboratory of Preclinical Pharmacology

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

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NIMH, NIH, Bethesda, Maryland 20205

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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 the neuropeptide diazepam binding inhibitor (DBI) and γ-amino-buturic acid (GABA) in central and peripheral nervous system was studied. DBI is a newly found polypeptide isolated from rat and human brain. The distribution of DBI in nervous system and also the co-localization of DBI and the major inhibitory neurotransmitter, the GABA, was studied.

The results of this immunochemical study show that DBI is located in selective neuronal populations of rat brain. In rat brain DBI immunoreactivity was high in the medial amygdaloid nucleus, many hypothalamic nuclei cortex and in various areas of hippocampus. Whereas low or virtually no DBI immunoreactivity was seen in corpus callosum, internal capsule, optic tract, fornix and granular layer of hippocampus. DBI was found also in peripheral nervous system i.e. in nerve fibers of adrenal medulla and sympathetic ganglia.

In the rat cerebral cortex DBI immunoreactivity was located in neurons that are not GABAergic, however, in the cerebellum and in hippocampus DBI might be present also in GABAergic neurons. In primary cultures of hippocampal cells stained with double immunofluorescence technique some cells revealed both DBI and glumatic acid decarboxylase (GAD) staining demonstrating the possibility of DBI and GABA in the same cells.

PHS 6040 (Rev. 1/84)

GPO 904-917

Project Description:

An endogenous polypeptide of rat brain has been identified that in μM concentration is capable of displacing 1,4-benzodiazepines and preferentially over the esters of the 3-carboxylic acid derivatives of β-carbolines from their specific binding sites. This polypeptide has been termed diazepam-binding inhibitor (DBI). It has been shown that DBI injected intraventricularly in rodents elicts proconflict response and antogonizes the anticonflict action of benzodiazepines. DBI has 104 amino acid recidues and has a molecular weight of 1.1 X 10^4 dalton. DBI contains at least two identical octadecapeptide recidues (OBN) and may function as a precursor for D-N. It has been shown that the DBI-like material detected in rat brain by the radioimmunoassay in unevenly distributed in various brain areas, but the exact DBI cell location remains to be established.

It has been suggested that DBI may function as physiological ligand for benzodiazepine recognition sites which similarly to β-carboline down regulates GABA receptor function. Since DBI coexist with some but not all GABAergic neurons it might modulate specific GABAergic synapses, which may account for differences in the pharmacological profile of DBI.

Rabbits were immunized with DBI purified to physical homogenelty from rat brain in complete Freund adjuvant. The anti-serum obtained had a high affinity for rat brain DBI. Under a standard radioimmunoassay conditions as little as 0.05 pmol of authentic DBI could be detected with the antiserum diluted 1;10000 and labelled DBI. The antiserum did not cross react with several other brain neuropeptides (VIP, ekephalins, dynorphin, substance P, somatostatin, ACTH, neurotensin, colecystokinin, myelin basic proteins, histones, GABA-modulin and MSH). Nitrocellulose electroblotting (Western blot) evinced a single band of DBI-like immunoreactivity and HPLC analysis produced a single peak of DBI immunoreactivity.

Using the DBI antiserum coupled with peroxidase reaction an intense immunoreactivity was detected in axins and cell bodies of many brain regions. A dense network of immunoreactivity was detected in many hypothalamic nuclei particularly in the arcuate nucleus, the corpus amygdaloideus, the hippocampus and the cerebellum. Immunoreactivity was less dense in the median eminence, straitum, cortex and thalamic nuclei. Low or absence of DBI immunoreactivity was seen in many brain areas i.e. corpus collosum, striatum internal capsule, fornix, mamillothalamic tract, optic tract and granular layer of hippocampus. In the cortex, DBI immunoreactivity was virtually absent in the external layers of cortex but was dense in pyramidal cells in layers 5 and 6. In the hippocampus DBI immunoreactivity was locating in a palisade cells in the subgranular zone and after colchicine treatment also in pyramidal cells of all CA areas.

The relatively low density of DBI containing neurons in brain areas such as the striatum and substantia nigra, which are rich in GABA containing neurons suggest that not all GABAergic cells may contain DBI. Morower pyramidal cells in the hippocampus and cortex which presumably do not contain GABA, shows strong DBI immunoreactivity. However, DBI locating in subgranular zone and in cerebellum is similar to the reported immunocytochemical localization of GABA. This raised the question as to weather DBI and GABA coexist in same neurons. For this purpose the primary cultures of hippocampal cells were used because the possibility of double immunostaining. These studies have shown that in some cells DBI and GABA coexist but in many cells no such coexistence is detectable. This may suggest that not all kthe GABAergic cells contain DBI or that the gene of the code for DBI are expressed differently in different GABAergic neurons.

In conclusion DBI is located in neurons of various hypothalamic nuclei and of the cerebral, cerebellar and limbic corticies which are important for control of emotion and anxiety. This new family of neuropeptides may have a physiological role in regulating the onset of behavioral patterns of typical conflict situations as anxiety and aggresion.

This study has not been terminated. The study of DBI in fine neuronal structures will be the next step. The study DBI-immunoreactivity location in human brains and the immunohistochemical study of the octadecapeptide are the logical steps scheduled in the investigation of the role of this peptide in neuronal function.

Publications:

Alho et al.: Diazepam-binding inhibitor: a neuropeptide located in selected neuronal populations of rat brain. Science, in press, 1985.

Alho et al.: γ-aminobuturic acid (GABA) in the adrenal medulla: location, pharmacology and function. In Neurohistochemical Today, Eds. S. Soinila and P. Panula, Alan R. Liss, NY 1985, in press.

Costa et al.: Cotransmission at GABAergic synapses. Stockholm Paper, in press, 1985.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-MH 01587-01 SMRP

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Dihydropyridines Block Calcium Uptake Into Cerebellar Granule Cells

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

P.I.: Ezio Carboni	Guest Researcher	SMRP NIMH
Other: Walter Wojcik	Senior Staff Fellow	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:	PROFESSIONAL:	OTHER:
1.0	1.0	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.)*

External calcium can enter a cell through the voltage sensitive calcium channel. In non-neuronal cells as well as peripheral nerves, the depolarization induced uptake of calcium through these channels can be antagonized with the class of drugs called dihydropyridines eg. nitrendipine. We have shown that calcium uptake into primary cultures of cerebellar granule cells can be inhibited by nitrendipine and stimulated by BAY K 8663, a dihydropyridine agonist at the voltage sensitive calcium channel. Thus, this research confirms the presence of a functional dihydropyridine, voltage sensitive calcium channel in cells from the central nervous system.

PHS 6040 (Rev. 1/84)

GPO 904-917

Project Description:

Calcium influx through a voltage dependent calcium channel is important in coupling excitation and contraction in cardiac and smooth muscle and in releasing neurotransmitters. In the last few years, the voltage sensitive calcium channel antagonists have been tested in the above systems. Even though they have recognized effects in cardiac and smooth muscle preparations, some controversy exists about their action in neuronal systems. In a brain membrane preparation, H-3 nitrendipine binding has been well characterized, demonstrating the presence of high affinity sites. Despite the presents of these high affinity binding sites, it has been difficult to show any antagonism of calcium uptake into neuronal tissue by the dihydropyridine antagonists as nitrendipine or stimulation of uptake by the agonist BAY K 8644.

We have investigated the voltage senstive calcium channels by measuring Ca-45 uptake and H-3 nitrendipine binding in primary cultures of cerebellar granule cells and in differentiated neuroblastoma-glioma, NG108-15 cells. In membranes prepared from eight day old cultures of granule cells, we observed a high affinity H-3 nitrendipine binding site. This was displaced by other dihydropyridines with half maximal inhibiting concentrations in the nanomolar range. In intact granule cells, nitrendipine was able to inhibit calcium uptake, using Ca-45 as a tracer, which resulted from depolarizing concentrations of potassium chloride, KCl (60mM), and veratridine, an activator of membrane sodium channels. The dihydropyridine agonist, BAY K 8644, stimulated calcium uptake when the cells where introduced to either 60 mM KCl and veratridine and nitrendipine antagonized this stimulation of calcium uptake. In NG108-15 hybrid cells, depolarizing concentrations of potassium were not effective in stimulating calcium uptake unless the cells were induced into a nondividing, neuron-like cell by prostaglandin E1. In the differentiated cells, the potassium induce calcium uptake was also antagonized by nitrendipine. Thus, it appears that these calcium channels are not just localized to peripheral tissues e.g. heart, skeletal muscle, and to periperhal nerves, but to central nerves as well. Our research confirms the existence of voltage sensitive calcium channels in cells from the central nervous system and directs future research into the regulation and physiological role of these calcium channels.

Because the entry of external calcium is required in the release mechanism of neurotransmitters, regulation of these channels by pharmacological means may prove to be therapeutically beneficial in regulating neuronal release. Recent observations show that the channel agonist BAY K 8664 is proconvulsant. The converse may also hold true that the antagonists may be anti-convulsant. In conclusion, we have only recently observed the dihydropyridine, voltage sensitive calcium channels to be functional in neurons.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-MH 01588-01 SMRP

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

GABA B Receptors Inhibit Adenylate Cyclase

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

P.I.:	Walter J. Wojcik	Senior Staff Fellow	SMRP NIMH
Others:	Jian Xu	Visiting Fellow	SMRP NIMH

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	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.)*

The activation of central GABA B receptors inhibits the formation of the second messenger, cyclic AMP, by acting through the inhibitory guanine nucleotide (Ni) unit of adenylate cyclase coupling mechanism. We studied the GABA B receptors which are present on cerebellar granule cells in primary culture. Our studies show GABA and baclofen, an analog of GABA, can attenuate basal and forskolin-stimulated adenylate cyclase activity in plasma membranes from these cells and can inhibit the forskolin-stimulated accumulation of cyclic AMP content in intact cerebellar granule cells. This inhibitory action to adenylate cyclase activity and cyclic AMP accumulation can be prevented by pretreating the cerebellar granule cells with a pertusis toxin termed islet activating protein (IAP). IAP reportedly affects the Ni unit so as to prevent the transfer of signal from the activated receptor to the catalytic unit of adenylate cyclase. Presently, we are testing whether this pretreatment with IAP acts by the ADP-ribosylation of the 41,000 dalton subunit of the Ni. Another indicator that the GABA B receptor couples with the Ni unit is that the half maximal concentration of GTP, needed to elicit the baclofen-mediated response to cyclase, is similar to the GTP concentration described for other receptor types coupled to the inhibitory adenylate cyclase. In summary, we have provided more evidence to support our hypothesis that the GABA B receptor directly couples to the Ni unit of adenylate cyclase.

PHS 6040 (Rev. 1/84)

GPO 904-917

Project Description:

The activation of GABA B receptors in the central nervous system of rat inhibits adenylate cyclase activity. Different degrees of inhibitory activity were observed in synaptosomal membranes prepared from various brain regions, correlating somewhat with the reported GABA B ligand binding activities. Our studies with neurologically mutant mice, which were deficient in certain cerebellar cell types, associated a loss of granule cells with a loss in the GABA B mediated inhibition of cyclase. Indeed, primary cultures of cerebellar granule cells contain the GABA B receptors which inhibit cyclase activity. Within the last ten years, various receptor types, e.g. cholinergic muscarinic, opiate delta, adenosine A1, somatostatin, etc, were reported to inhibit the formation of cyclic AMP. Each receptor type has been shown or is believed to mediate their inhibitory action on adenylate cyclase through the Ni unit present in plasma membranes. We hypothesized that the GABA B receptor, similarly, couples to the Ni unit. Initially, we have shown that GTP is required for the GABA B receptor-mediated inhibition of cyclase and that the half maximal concentration (Km) for GTP was approximately one-half micromolar. This Km is similar to that reported for the other receptors inhibitory to cyclase and is about ten times greater than the Km for GTP needed at those receptors stimulatory to adenylate cyclase. In the transfer of the signal from the agonist, the agonist-receptor complex must stimulate the hydrolysis of GTP to GDP (GTPase) on the Ni unit. The GTPase can be inhibited by a pretreatment with IAP which ADP-ribosylates the Ni unit. Thus, antagonizing the agonist induced inhibition of cyclase at the level of the Ni unit. An IAP pretreatment of the cerebellar cells in culture results in a loss of the baclofen mediated inhibition. This is seen in both an in vitro/membrane preparation and in an in vivo/intact cell procedure. Presently, we are in the process of verifying whether the IAP pretreatment has ADP-ribosylated the Ni unit. An in vitro ADP-ribosylation with IAP is performed on membranes from either the IAP pretreated or the untreated cerebellar granule cells. This procedure should show a positive ADP-ribosylation in the untreated and no ADP-ribosylation in the previously IAP-treated cells. Overall, these findings strongely support the view that the GABA B receptor belongs to the recently described class of receptors which are inhibitory to adenylate cyclase. Our present interests are directed toward the physiological function of the GABA B receptor. We speculate that it's role is to regulate or attenulate protein phosphorylation by a cyclic AMP dependent phosphorylation. We are also interested in whether the GABA B agonists affect the uptake of extracellular calcium and whether calcium uptake is modified by phosphorylation through a cyclic AMP dependent protein kinase.

In cerebellum the GABA B receptor is believed to be associated with nerves, such as the glutamatergic cerebellar granule cells, and more specifically with the nerve terminals of these cells. It appears that the function of this receptor and others which are coupled to the inhibitory adenylate cyclase is one of regulating excitatory transmitter release. Baclofen, a GABA B analog, has anti-spastic activity in individuals with multiple sclerosis and spinal cord injury. This drug is thought to block the mono-and poly-synaptic muscle reflex pathway at the level of the spinal cord by preventing the release of excitatory transmitter such as substance P from the primary afferent nerves. It is also possible that baclofen could have other therapeutic actions as an antiepileptic, analgesic and sedative. These actions of baclofen are also observed with other agonists at receptors which are coupled to the inhibitory adenylate cyclase and the effectiveness of one receptor agonist over another could reside in their different regional distribution in brain.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-MH 01589-01 SMRP

PERIOD COVERED

October 1, 1984 through September 30, 1985

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

Target - Derived Neuronotrophic Factors

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

Bradley Wise, Staff Fellow, Laboratory of Preclinical Pharmacology; Michel Emerit, Visiting Fellow, Laboratory of Preclinical Pharmacology; Hannu Alho, Visiting Associate, Laboratory of Preclinical Pharmacology

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Molecular Neurobiology

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.9	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.)*

Nerve growth factor is a target-derived protein for the peripheral sympathetic and sensory nervous system. Similar trophic factors have been postulated to exist for central nervous system neurons. The identification of a neurotrophic factor involved in the cholinergic septo-hippocampal pathway is the subject of this research project. Conventional and molecular biological approaches to this problem have been initiated. Primary dissociated cultures of rat fetal septal area neurons have been established as an assay system to monitor the activity of the cholinergic trophic factor. These cultured cells have an uptake system for choline that is time -, temperature -, and partially Na - dependent. Preliminary results also show that acetylcholine is synthesized from the choline taken up by the cells. Thus, septal cells in culture are a suitable system to assay for cholinergic trophic factor (s) that may be induced by lesions of the septo-hippocampal pathway. A molecular biological approach is also being used to identify and, ultimately, characterize the trophic factor. Complementary DNA (cDNA) has been synthesized from hippocampal mRNA and will be used in cDNA-mRNA hybridizations to identify lesion-induced and novel mRNA species. Injection of frog oocytes with mRNA fractions will serve as an in vitro translation system and the encoded trophic proteins will be assayed by alterations in the cholinergic parameters of the cultured septal cells. Identification and cloning of a novel cholinergic neuronotrophic factor may lead to new therapeutic approaches to neurological diseases with an altered cholinergic component, such as Alzheimer's disease.

PHS 6040 (Rev. 1/84) GPO 904-917

Project Description:

Objectives: Nerve growth factor is a target-derived protein trophic factor for the peripheral sympathetic and sensory nervous system. Similar trophic factors have been postulated to exist for central nervous system (CNS) neurons. The goal of this research project is to identify and characterize a postulated cholinergic neuronotrophic factor. The predominately cholinergic septo-hippocampal pathway was chosen as the model to study CNS target-derived (i.e. hippocampal) trophic factors. Fimbria-fornix lesions of the pathway are known to decrease cholinergic activity in the target hippocampus, and so may induce the synthesis of a cholinergic neurotrophic factor.

In order to identify the factor in hippocampal extracts and to follow the activity during purification, a suitable assay system had to be established. We have set up primary cultures of dissociated neurons from the septal area of fetal (embryonic day 17) rat brains. The cells were maintained in serum-free culture conditions for up to 10 days. Immunocytochemical evidence indicates that about 5% of the cultured cells are astrocytes (positive staining for glial fibrillary acidic protein) and the remainder are neuronal cells (positive staining for neurofilament protein). Cells maintained in culture for 6 days take up [^{3}H] choline chloride at 0.1 and 1 μM concentrations in a time and temperature dependent process. Uptake of choline in the presence of Na^+ was about twice the uptake under Na^+- free conditions, indicating the Na^+-dependent nature of at least part of the uptake system. Further studies will clarify whether the choline uptake in these cells is by the high-affinity choline uptake system that is associated with cholinergic neurons. Preliminary evidence indicates that these cell cultures synthesize acetylcholine from the [^{3}H] choline precursor. Other biochemical parameters of the cholinergic neuron, such as the presence of choline acetyltransferase and the in site synthesis of acetylcholine, will be further studied to assess the cholinergic nature of these cells and to assess the role of trophic factors in the survival and development of these cells in culture. Alterations in the expression of these various cholinergic parameters by hippocampal extracts derived from normal and lesioned (i.e. fimbria-fornix lesioned) rats will be used as an index for the presence of a cholinergic trophic factor.

A molecular biological approach to this problem is also being initiated. Messenger RNA (mRNA) has been isolated from rat hippocampus and complementary DNA (cDNA) was synthesized from the mRNA template by Dr. M. Emerit. The cDNA will be used in cDNA-mRNA hybridizations to identify those mRNAs that are induced as a result of septo-hippocampal lesions. The hippocampal mRNA used for these hybridizations will be partially purified by Dr. Emerit using sucrose density gradient centrifugation or agarose gel electrophoresis. The mRNA fraction encoding the cholinergic trophic factor will be identified by micro-injection of mRNA into frog oocytes and assaying the synthesized proteins for trophic activity using the primary cultures of septal cells as a bioassay. The active mRNA fraction from control and lesioned rats can be used in the cDNA-mRNA hybridizations to further enrich for those mRNAs that are induced by the lesion. The purified mRNA population can then be used to screen a cDNA clonal library or used to generate a cDNA library of lesioned-induced mRNAs. The ultimate goal is to obtain a cDNA clone for the cholinergic neurotrophic factor.

The importance of this research project is clear when one considers the involvement of the cholinergic system in certain neurological diseases, such as Alzheimer's disease. The etiology of Alzheimer's disease is unknown but a specific degeneration of cholinergic neurons in the cortex of the brain is one manifestation of the disease state. It is quite possible that a deficiency in a cholinergic factor or an altered structure of the factor may at least be one contributing cause of the disease. Identification, purification and isolation of a cDNA clone for the neurotrophic factor involved in the cholinergic neuronal system may lead to novel diagnostic and therapeutic interventions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-MH 01590-01 SMRP

PERIOD COVERED

October 1, 1984 through September 30, 1985

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

Origin of FMRF-NH_2 Immunoreactivity in Rat Spinal Cord

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

Carlo Ferrarese, Visiting Associate, Laboratory of Preclinical Pharmacology

Dr. Michael Iadarola, Staff Fellow, Laboratory of Preclinical Pharmacology; Dr. Hsui-Ying Yang, Pharmacologist, Laboratory of Preclinical Pharmacology

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Section on Neuropeptides

INSTITUTE AND LOCATION

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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.)*

Phe-Met-Arg-Phe-amide immunoreactivity (FMRF-NH_2-IR) is highly concentrated in the dorsal horn of rat spinal cord, and particularly in nerve terminals of lamina I. In order to establish the location of the cell bodies of the lamina I terminals containing FMRF-NH_2-IR in sensory ganglia and in spinal roots. FMRF-NH_2-IR was found in both tissues, and reverse-phase HPLC analysis revealed that both tissues contain the same molecular forms that are also present in the spinal cord. Lumbo-sacral rhizotomy, induced a 50% decrease of FMRF-NH_2-IR in the lumbar segment of the spinal cord, suggesting that at least a portion of the FMRF-NH_2-IR present in this tissue is of peripheral origin. Transection of the spinal cord at the midthoracic level induced a 20 to 50% decrease of FMRF-NH_2-IR in the lumbar segment of the spinal cord, suggesting also that the presence of FMRF-NH_2-IR in descending pathways.

PHS 6040 (Rev. 1/84)

GPO 904-917

Objectives: FMRF-NH_2 I.R. is highly concentrated in dorsal horn of rat spinal cord, in lamina I nerve terminals. Chromatographic analysis revealed that this I.R. is different from FMRF-NH_2 isolated from clam because immunoreactivity resides in larger molecular weight peptides, with different chromatographic properties. The function of these peptides is still unknown, but they seem to act as outacoids modulating opiate action.

For a better understanding of the physiological role of these peptides, we tried to determine whether nerve terminals staining for FMRF-NH_2 I.R. originate from cell bodies located in spinal cord. We directed our attention to these terminals because this CNS area is involved in gate control of afferent sensory pathways, and because in this area neurons containing opioid peptides are located.

To gain information on the location of the cell bodies containing FMRF-NH_2 I.R. was studied whether FMRF-NH_2 I.R. is present in sensory ganglia and in spinal roots. In addition we studied the effects of lumbo-sacral rhizotomy and spinal cord transection on FMRF-NH_2 I.R. in different segments of spinal cord.

Method Employed: Lumbo-sacral rhizotomy was performed through a laminectomy at L_3-L_4 vertebrae; cauda equina was exposed and dissected at this level.

Spinal cord transection was performed through a laminectomy at T_5-T_6 vertebrae.

Peptide detection and measurments were carried out on cervical and lumbar segments of spinal cord. These were removed and dissected on ice into dorsal and ventral halves. Roots and sensory ganglia were subsequently collected. Tissues were homogenized in 1N acetic acid - 0.02 N HCl - 0.1% beta-mercaptoethanol. After centrifugation aliquots of supernatants were dried and used for FMRF-NH_2 RIA.

Reverse phase HPLC analysis of tissue extracts was performed with a linear gradient from 20 to 60% of acetonitrile.

Results: FMRF-NH_2 I.R. was found in sensory ganglia (180 $\pm$ 16 fmol/mg prot) and in spinal roots (36 $\pm$ 4 fmol I/mg prot). HPLC analysis revealed in both tissues the same molecular forms of spinal cord.

In order to confirm that part of spinal cord FMRF-NH_2 I.R. is originating from sensory ganglia, we performed lumbo-sacral rhizotomy. One week after this surgical treatment we found a 50% decrease of FMRF-NH_2 I.R. in lumbar segment of spinal cord, compared to cervical segment of the same animals and to lumbar segment of control animals. To examine the possible presence of FMRF-NH_2 I.R. in nerve fibers descending from supra spinal centers to dorsal horn of spinal cord, we performed spinal cord transection at midthoracic level. This surgical treatment induced a decrease of FMRF-NH_2 I.R. in lumbar cord: 20% after 1 week and 50% 30 days after transection. HPLC analysis revealed a reduction of the amounts of all peaks, suggesting that all the molecular forms of FMRF-NH_2 I.R. are also descending from supraspinal centers.

Significance for biomedical research. Peripheral and central FMRF-NH_2 I.R. can cooperate at spinal cord level in modulating opiate actions. They also can be involved in mechanism of tolerance.

Proposed course: Since FMRF-NH_2-like-immunoreactivity is present in an octa and actadeca neuropeptide both containing the carboxyterminal P5o-Gln-Phe-Arg-NH_2 the pharmacological profile of this tetrapeptide will be determined with regard to action on pain threshold, antagonism to morphine antinociception and action on delta receptors function present in specific neuroblastoma cultures containing opiate receptors. Specific binding of the tetrapeptide to these cells and to synaptic membranes will be studied. This study will be directed to characterize whether Pro-Gln-Phe-Arg-NH_2 is endowed with the capacity to modify endogenous peptide action.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01591 SMRP

PERIOD COVERED

October 1, 1984 through September 30, 1985

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

Abnormailty of α- Adrenergic Receptors in the Frontal Cortex of GEPR

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

Ferdinando Nicoletti, M.D., Guest Researcher, Laboratory of Preclincal Pharmacology

Michael Iadarola, Staff Fellow, Laboratory of Preclincal Pharmacology; M. Barbaccia, Guest Researcher, Laboratory of Preclincal Pharmacology; H.E. Laird

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Neuropeptides

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

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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.)*

We have found that the binding site of ^{3}H-prazosin, a selective ligand for α_1-adrenergic recognition sites is significantly lower in the frontal cortex of the genetically epilepsy prone rats (GEPR), as compared with normal Sprague Dawley rats. Scratched analysis reveals a decrese in the Bmax of ^{3}H-prazosin binding with no change in the apparent K_D, suggesting that there are fewer α_1-adrenergic recognition sites in the frontal cortex of the GEPR. This abnormality is associated with a reduced capacity of norepinephrine (NE) to stimulate ^{3}H-inositol-1-phosphate formation in frontal cortex slices prelabeled with ^{3}H-inositol. No significant differences in ^{3}H-prazosin binding as well as NE-stimulated ^{3}H-inositol-1-phosphate formation have been observed in other brain regions including hippocampus, corpus striatum and inferior colliculus. These results indicate that a deficit in the α_1-adrenergic receptor system in the frontal cortex may play a role in the seizure process in the GEPR.

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In order to characterize the nature of the noradrenergic abnormalities in Genetically Epilepsy Prone Rats (GEPR), we have measured α-adrenergic receptor binding in the frontal cortex and hippocampus of these animals using 3H-prazosin and 3H-clonidine as selective ligards for α_1 and α_2 adrenergic receptors, respectively. In addition, the stimulation of inositol phospholipid hydrolysis has been measured in slices of brain regions as a functional index of α_1-adrenergic receptor activation.

We have found that, in GEPR, both the x_1 adrenergic receptor binding and the stimulaion of inositol phospholipid hydrolysis induced by norepinephrine are markedly reduced in the frontal cortex but not in other brain regions. No significant changes in α_2-adrenergic receptor binding were found in GEPR. We suggest that a deficit in the α-adrenergic receptor system in the frontal cortex may play role in the seizure process of the GEPR.

Methods Employed: a) The density and the dissociation constant of α_1 and α_2 receptors were determined by measuring the specific binding of 3H-prazosin and 3H-clonidine to crude synaptic membranes from discrete brain regions; b) the hydrolysis of membrane inositol phospholipid was determined by measuring the accumulation of the product inositol-mono phosphate in slices stimulated with norepinephrine.

Proposed Course: a) study on the mechanisms underlying the "seizure-prone" status of GEPR; b) correlation of α_1 receptor function and inositol phospholipid metabolism with altered statu of brain function.

Publication:

F. Nicoletti, M.J. Barbaccia, M. Iadarola, O. Pozzi and H.F. Laird II: Abnormality of α_1 adreneric receptors in the frontal cortex of epileptic rats. J. Neurochem., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01592 SMRP

PERIOD COVERED

October 1, 1984 through September 31, 1985

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

Studies on an Anxiogenic Neuropeptide (DBI) in Human Brain and Cerebrospinal Fluid

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

M. L. Ferrero, Guest Worker, LPP

M. L. Barbaccia, Guest Worker, LPP; D. Pickar; S. Paul; F. K. Goodwin; A. Guidotti, Section Chief, Lpp; E. Costa, Lab. Chief, LPP

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

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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.)*

DBI is a neropeptide isolated from rat and human brain which interacts with beta-carbolines and benzodiazepine recognition sies and down regulates GABA receptor function. Using a specific antibody raised against human DBI we have measured DBI content in the cerebrospinal fluid (CSF) of 28 normal volunteers age and sex matched with a group of 45 patients suffering from various neuropsychiatric disorders. The amount of DBI-like immunoreactivity in CSF appears to change with age, sex and in a small group of depressed patients. However, conclusion that changes of CSF-DBI content are related to psychiatric symptoms requires a greater number of cases to be analyzed.

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GPO 904-917

Description:

DBI is a neruopeptide purified from rat brain homogenates which displaces beta-carbolines form specific brain binding sites. When injected intraventricularly into rats, DBI has a pharmacological profile similar to that of anxiogenic beta-carbolines. Studies with tryptic DBI fragments suggest that the active portion of DBI molecule is contained in an octadecaneuropeptide (ODN) with the following amino acid sequence:
Gln-Ala-Thr-Val-Gly-Asp-Val-Asn-Thr-Asp-Arg-Pro-Gly-Leu-Leu-Asp-Leu-Lys
(neuropharmacology 23: 1359, 1984).

The aim of this study was to study the presence and changes of DBI in brain and CSF of human subjects.

Major Findings:

Working with postmortem human brain extracts, we have now purified a neuropeptide which appears to be similar to rat DBI with respect to molecular weight (aprox. 11,000) amino acid composition and retention time on reverse phase HPLC. Similarly to rat DBI, the neuropeptide extracted from human brain elicits proconflict responses when injected intracerebroventricularly in thirsty rats. Human DBI cross-reacted with an antibody raised against synthetic ODN with a potency similar to that of rat DBI. Moreover, by sequencing various tryptic fragments of human DBI, we detected the ODN sequence found in rat DBI. However, studying a number of antisera raised in rabbits against human and rat DBI, we found a poor cross reactivity (1/1000) betwen the rat and human neuropeptides. Using a specific antibody raised against human DBI we have measured neuropeptides. Using a specific antibody raised against human DBI we have measured DBI content in a few areas of postmortem human brain and found a regional distribution (amygdala cerebeluum hippocampus corte striatum) similar to that observed in rat. Moreover DBI-like immunoreactivity was found in human spinal fluid.

The CSF studies were carried out in a group of 28 normal volunteers, age and sex matched with a group of 45 patients suffering from various neuropsychiatric disorders. Only 100 μ l of CSF are needed for a reliable quantitation of DBI-like immunoreactivity with our antiserum. The specificity of this assay was determined by immunoblotting and HPLC. For routine assay CSF was acidified (1 N acetic acid) and 100 μl aliquots of the extract was tested for competition with ^{125}I-Bolton-Hunter reagent labeled DBI. The displacement of ^{125}I DBI by increasing concentrations of CSF extract shows a slope identical to that of authentic DBI. The DBI stability of CSF was tested by measuring DBI-like immunoreactivity in aliquots of the same smple after different treatments: 1) heating 15 minutes at 90°;2) acidification with 1 N acetic acid and heating; 3) acidificiation with 0.1 N and 1.0 N acetic acid; 4) freezing and thawing; 5) incubation for up to 24 hours at room temperature. The highest content of DBI-like immmunoreactivity was found in acidified samples. Serial measurements of DBI-like immunoreactivity in the same samples yielded virtually the same amounts. Moreover, when serially collected fractions of CSF from normal volunteers were assayed virtually no concentration gradient of DBI-like immunoreactivity was found. Pooling together the 73 samples, the amount of DBI-like immunoreactivity in CSF appears to change with age (aged young) and sex (male female). We have indications of modifications in CSF-DBI content related to psychiatric symptoms, but at this time, we refrain to make any conclusion waiting for a greater number of cases to be analyzed.

The data suggest that human and rat DBI though similar may not be identical, immunologically. However since similarly to rat DBI, human DBI causes a proconflict action in rats when injected intraventricularly and since they both contain the ODN sequence we believe that human and rat DBI are equivalent functionally. Moreover the presence of high concentrations of DBI in selected areas of human brain and in spinal fluid allows to infer that this neuropeptide may play an important role in the manifestation of anxiety and other symptoms of various neuropsychiatric disorders.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01593-01 SMRP

PERIOD COVERED

October 1, 1984 through September 30, 1985

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

Excitatory aminoacid receptor function in cerebellar granule cells

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

F. Nicoletti, M.D., Guest Researcher, Laboratory of Preclinical Pharmacology

J.T. Wroblewski, Guest Research, Laboratory of Preclinical Pharmacology

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Neuropeptides

INSTITUTE AND LOCATION

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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.)*

We have used primary cultures of cerebellar granule cells to study the intracellular molecular processes elicited by the interaction of excitatory amino acids with specific membrane receptors. We have shown that at least two different dicarboxylic amino acids receptors are coupled with inositol phospholipid metabolism in primary cultures of cerebellar granule cells; one is sensitive to the stimulatory action of glutamate and aspartate and is preferentially antagonized by 2-amino-5-phosphonovalerate (APV); the other is activated by kainic acid and preferentially antagonized by cis, 2,3-piperidin-dicarboxylic acid (PDA). The activation of these sites by glutamate, asfertate and kainic acid also results in an enhanced formation of cGMP. The stimulatory action of glutamate and aspartate on cGMP is markedly influenced by the ionic composition of the incubation buffer and is potentiated by depolarizing stimuli. The action of kainic acid is independent of such parameters.

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The function of dicarboxylic amino acid receptors has been previously studied in brain slices or synaptosomes. In our study, the use of primary cultures of cerebellar granule cells eliminates the glial compartment (as opposed to brain slices) and includes the postsynaptic neuronal membranes, preserving the functional integrity of the cell (as opposed to synaptosomal preparation).

A) Turnover rate of membrane inositol phospholipids. We have shown that dicarboxylic amino acids stimulate inositol phospholipid hydrolysis in primary cultures of cerebellar granule cells with the following order of potency: glutamic acid > quisqualic acid > kaimic acid > aspertic acid = ibotenic acid > NMDA = quinolinic acid.

The stimulatory action of glutamic acid on inositol phospholipid hydrolysis is preferentially antagonized by 2-amino-5-phosphonovaleric acid (APV) whereas the stimulation by kainic acid is antagonized by cis-2,3-piperdindicarboxylic acid (PDA). Hence, we conclude that at least two different dicarboxylic amino acid receptors are coupled with inositol phospholipid metabolism in primary cultures of cerebellar granule cells.

B) Study on cyclic GMP (cGMP)

We have characterized pharmacologically and functionally the stimulatory action of dicarboxylic amino acids on cyclic GMP formation in primary cultures of cerebellar granule cells.
The stimulatory action of glutamic, aspartic acids and NMDA on cyclic GMP is dependent on the ionic composition of the incubation buffer. The absence of Mg^{2+} or the reduction of Cl- ion concentration in the incubation buffer markedly enhance the stimulation of cGMP by glutamic, aspartic acids or NMDA. Similar results were obtained when granule cells were incubated in the presence of dicarboxylic amino acids under depolarizing stimuli (i.e. effective concentrations of veratridine).

By contrast, the stimulatory action of kainic acid on cGMP formation has been found to be independent of the ionic composition of the incubation buffer as well as by veratridine - induced changes in membrane potential. The response to glutamic and aspartic acids and to kainic acid can be also differentiated pharmacologically: APV preferentially antagonizes the increase in cGMP induced by glutamic acid, whereas PDA preferentially antagonizes the action of kainic acid.

Methods Employed:

Primary cultures of cerebellar granule cells were obtained from 8 day old rats. The turnover rate of inositol phospholipid was estimated either by measuring the rate of accumulation of inositol phosphates in the presence of Li^{+} or by measuring the rate of disappearance of membrane phosphoinositides by thin layer crowatography. cGMP levels were measured by RIA.

Proposed Course: a) Study on the molecular process involved in the coupling between dicarboxylic amino acid receptors activation and guanylate cyclase stimulation; b) Interaction between stimulation of inositol phospholipid hydrolysis, protein kinase C and guanylate cyclose activation in the intracellular response to excitatory amino acids.

Publications:

F. Nicoletti, J.T. Wroblewski, A. Novelli, H. Alho, A. Guidotti and E. Costa: Activation of inositol phospholipid metabolism is a signal transducing system for dicarboxylic excitatory amino acids. Submitted to J. of Neuroscience.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01594-01 SMRP

PERIOD COVERED

October 1, 1984 through September 30, 1985

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

Excitatory amino acid receptors are coupled with phosphoinositide metabolism

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

Ferdinando Nicoletti, M.D., Guest Researcher, Laboratory of Precl. Pharmacology

J. Wroblewski, Guest Researcher, Lab. of Preclin. Pharm.; Michael Iadarola, Staff Fellow, Lab. of Preclin. Pharm.

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Neuropeptides

SECTION

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INSTITUTE AND LOCATION

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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.)*

We have shown for the first time that specific dicarboxylic amino acid recognition sites are coupled with inositol phospholipid metabolism in the rat central nervous system. These sites are abundant in the hippocampus, sensitive to the stimulatory action of ibotenic acid and are selectively antagonized by 2-amino-4-phosphonobutyric acid (APB). Glutamic and aspartic acids have marginal effect on inositol phospholipid metabolism in slices from adult animals but they markedly enhance inositol phospholipid hydrolysis (up to 15 fold) in slices from newborn animals. This action of glutamic acid declines progressively from the day 6th to the day 19th after the birth up to reach the adult levels on the day 30th after the birth.

In the adult, the stimulatory action of dicarboxylic amino acids on inosidol phospholipid hydrolysis is potentiated following repeated electrical stimulation of limbic structures or after toxin-induced lesions of hippocampal excitatory pathways.

We suggest that the stimulation of inositol phospholipid hydrolysis elicited by dicarboxylic amino acid may have a role in the cellular mechanism(s) underlying neuronal development as well as the regulation of limbic excitability.

PHS 6040 (Rev. 1/84)

GPO 904-917

Project Description: We have shown for the first time that a specific dicarboxylic amino acid recognition site is coupled with inositol phospholipid metabolism in the rat central nervous system. This site is abundant in the hippocampus, is present in corpus striatum, hypothalamus, cerebellum and frontal cortex and is virtually absent in the spinal cord. Dicarboxylic amino acids enhance inositol phospholipid hydrolysis with the following order of potency: ibotenic acid > quiqualic acid ≥ and homocysteic acid > glutamic acid = aspartic acid. Kainic acid and NMDA fail to stinulate insitol phospholipid hydrolysis. The action of dicarboxylic amino acids on inositol phospholipids turnover rate is potently and selectively antaagonized by 2-amino-4-phosphonobutyrric acid (APB), a selective ligand for the Ca^{2+}-dependent glutamic acid binding.

Glutamic acid aspartic acid induce only minor changes in inositol phospholipid hydrolysis in hippocampal slices from adult animals but they have consistent effects (they increase inositol—phosphate formation by 12-15 fold) in slices from newborn animals. The potency of glumatic and aspartic acids declines progressively from the 6th to the 19th day after the birth up to reach the adult levels on the 30th day after the birth.

In adult animols, the stimulatory action of ibotenic acid and glumatic acid on inositol phospholipid hydrolysis is significantly enhanced following repeated electrical stimulation of limbic regions (hippocampus or amygdola) or following lesions of hippocampal excitatory pathways resulting from local infusion of colchicine or kainic acid. These results show that excitatory amino acid receptors are coupled with inonitol phospholipid metabolism in the central nervous system and suggest that the stimulation of inositol phospholipid hyoholysis by dicarboxylic amino acids may have a role in the cellular and synaptic mechanisms underlying development as well as changes in limbic excitablity.

Methods Employed: Inositol phospholipid hydrolysis has been estimated by measuring the accumulation of the phosphoinositide hydrolysis product inositol-1-phosphate (IP) in the presence of Li^+,in slices prelabelled with ^3H-inositol. ^3H-I-P has been isolated by amon exchange chromatography.

Results obtained have been confirmed by measuring the rate of disappearance of ^{32}P-labelled membrane inositol phospholipids by thin layer cromatography.

Proposed course: a) role of glutamate - stimulated phosphotidylinositol hydrolysis in the development process of hippocampal neurons; b) functional interaction between glutamate, aspartate and peptides (like "," or "k" opiate receptor agonists) in the central nervous system; c) use of brain tissue from newborn animals as a tool to characterize the intracellulor response to the stimulation of excitatory amino acid receptors.

Publications:

F. Nicoletti, J.L. Meek, M. Iadarola, D.M. Chuang, B.L. Roth and E. Costa; Coupling of inositol phospholipid metabolism with excitatory amino acid recognition site in rat hippocampus. J. Neurochem., in press.

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