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The College on Problems of Drug Dependence, Inc.

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Problems of Drug Dependence, 2000:
Proceedings of the 62nd Annual Scientific Meeting, The College on Problems of Drug Dependence, Inc.

Editor:

Louis S. Harris, Ph.D.
Virginia Commonwealth University

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INTRODUCTION OF DR. NORMAN MALDONADO, PRESIDENT UNIVERSITY OF PUERTO RICO

L. S. Harris

Medical College of Virginia of Virginia Commonwealth University, Richmond, VA

It gives me great pleasure to introduce an old and good friend, Dr. Norman Maldonado, who will bring us greetings from the Commonwealth of Puerto Rico. Dr. Maldonado, a distinguished scientist, teacher, and administrator is President of the University of Puerto Rico. The university is comprised of 11 campuses and highly regarded schools of medicine, law architecture and engineering, whose >500 faculty serve over 70,000 students. Needless to say, the University of Puerto Rico is the largest minority and Hispanic university in the United States.

We first met in 1975, during his long tenure as Chancellor of the University of Puerto Rico Medical Sciences Campus, where he did a superb job of developing the campus to a leading position as a provider of scientists and health care professionals to the countries of the Caribbean and Central America. His careful fostering of the Caribbean Primate Research Center, one the oldest and most productive primate centers, is recognized and appreciated by primatologists all over the world.

Dr. Maldonado serves or has served on numerous boards and commissions at both the Commonwealth and national levels. This includes his appointment by Secretary Shalala to the NIH Advisory Board of the National Center for Research Resources and his appointment by President Clinton to the Board of Directors of the Harry S. Truman Foundation.

On a more personal note, Dr. Maldonado is a humane and caring individual with a strong interest in the arts, music and Puerto Rican culture. He is also a devoted family man, which I am sure, will be attested to by his wife Mary Ann and his five children and three grandchildren.

It is my pleasure to present Dr. Maldonado.

WELCOMING REMARKS

N. I. Maldonado

University of Puerto Rico, San Juan, Puerto Rico

On behalf of the University of Puerto Rico, I welcome this distinguished group of dedicated professionals who are actively involved in the different areas of research, treatment, and prevention of problems associated with drug abuse. Our University is the public institution of higher learning in Puerto Rico: a complex system of 11 institutional units of significant diversity that includes colleges with two and four year programs leading to associate and bachelors degrees, as well as highly specialized campuses such as Río Piedras, Mayaguez, and the Medical Sciences Campus, which award doctoral degrees and are engaged in important research projects. The university manages under special arrangement, other units such as the University Hospital and three Diagnostic Centers.

The University of Puerto Rico was established in 1903, as a Teachers College to serve an urgent need for qualified teachers. Shortly thereafter, it expanded its offerings to include the liberal arts and other professional programs. In 1911, the College of Agriculture was founded as a separate campus in Mayaguez. Professional programs such as the School of Law and the School of Pharmacy were established in the main campus in Río Piedras in response to the people who demanded training in these key areas. Throughout its history, the institution has been strongly committed to the needs of the people, a fact witnessed by its process of growth and development.

The other institutional units date back to more recent decades, when the facilities of the main campuses at Río Piedras and Mayagüez were not able to accommodate an increasing number of applicants. Originally, junior colleges were established. Strategically located throughout the island, their original intention was to offer the first two years of the liberal arts and other professional two-year programs. By and large, the academic offerings were limited to programs leading to an associate degree. In the beginning, these units resembled community colleges. Eventually, they evolved into autonomous colieges, increasing their academic offerings according to the demand of the various regions, which they serve.

When these campuses were first established, they were located in areas that were either suburban or rural. With the growth of the nearby of towns and cities and the unavoidable encroaching development, however, many of them are now in the midst of ever expanding populated areas. This has meant that the urban environment of the sites has changed. Needless to say, such changes have made the campuses increasingly vulnerable to the problems inherent to their surroundings.

The large number of persons that can gather at any of our campuses at any given time makes them a target for persons of delinquent behavior who came from the immediate urban context. In some cases, they are street people who wander into the campus. Sometimes, however, they intrude upon the campus with the deliberate intention of carrying out their activities there, taking advantage of the great number of people that are found on premises. Others attend the various events, which are open to the community. This, clearly, represents a problem for the institutional units since the social problems of the community at large intrude upon the campuses. As an example, I would mention that although the delinquent activity that takes place on the campuses would be statistically classified as burglary, most are actually a result of the drug problems of the surrounding neighborhoods.

Needless to say, in strict compliance with government norms, we have an institutional policy regarding substance abuse in general and the use of controlled substances in particular. In addition to this, as part of our student's services, we offer orientation, guidance, counseling, and several additional professional services that include professional psychological and psychiatric help for those students in need of assistance, hoping that we can contribute in an effective way to their rehabilitation.

In August of 1997, the Quality of Life Program was created in the 11 institutional units of the University of Puerto Rico. This program was implemented to raise awareness and educate the campus community about wellness, healthy lifestyles, drug and alcohol prevention, crime prevention, and sexual assault. The Quality of Life Offices are responsible for assuring compliance with several federal government programs. One of the most emphatically underlined priorities of the program is the one dealing with alcohol and drug prevention. Innovative educational and prevention strategies are being implemented in this problem area.

The university promotes peer education as a useful tool in spreading prevention messages on alcohol use and drug abuse in its campuses as well as in high schools. It believes that students play an important part in encouraging peers in developing health life styles. The Quality of Life coordinators participate in the National Meeting on Alcohol, other Drug, and Violence Prevention in Higher Education, an annual activity that allows participants to share new strategies on prevention of abuse of alcohol and other substances.

Due to the nature of these problems related to drug use and dependence, there is an increasing need for activities that bring together all types of professionals in fields that deal with this problem. We are all aware that drug dependency is a major concern our society confronts. Whatever action is taken to help solve this problem will be a contribution to our society.

These are pressing and important matters you are dealing with. I wish you a very successful meeting as you discuss all viable alternatives in seeking solutions to the enormous problem of drug dependence.

Thank you.

INTRODUCTION OF THE NATHAN B. EDDY MEMORIAL AWARD RECIPIENT

B. R. Martin

Medical College of Virginia of Virginia Commonwealth University, Richmond, VA

This is truly an honor to introduce this year's recipient of the Nathan B. Eddy Memorial Award. It is also fitting that this award is made last because much of what has been said today is a recitation of the Eddy Award. To reiterate the introduction by our President, Dr. Michael Kuhar, it represents an individual who has made life-long contributions to the field. Actually, the criteria of the award are quite general. In thinking about the attributes of the recipient to emphasize today, I examined the list of past awardees, for it is these individuals who best define the Nathan B. Eddy Memorial Award. These individuals have made life-long contributions to our field. These are the leaders who have made an impact, these individuals who have made a difference. It is easy to identify some aspect of their contribution that has changed our field, whether it is outstanding scientific contributions, mentoring young scientists or taking a leadership role. This year's recipient, Dr. William L. Dewey, meets all of these criteria. He is an individual who has made a difference and continues to do so. I would like to state that it was Dr. Horace Loh who initiated the nomination process for Bill. I agreed with Horace who said, "Now is the time, we should have done it earlier."

As I had never met Dr. Eddy, I asked Dr. Louis Harris whether Dr. Eddy and Bill shared some common traits. Lou very quickly replied that Dr. Eddy was very interested in understanding the mechanism of actions of drugs of abuse, particularly as they related to drug addiction. Moreover, Dr. Eddy had a desire to translate these findings in ways that would impact the ills produced by drugs of abuse on society. These are the motivations of Bill Dewey. Lou also stressed that Dr. Eddy was keenly interested in being a mentor and developing the careers of other individuals, goals that are shared by Bill Dewey.

I would like to elaborate very briefly about some of the commonalties between Dr. Eddy and Bill. I will not discuss in any depth his research, because he is going to review a little bit with several hundred slides. While it is not always easy to fully gauge someone's total impact on a scientific field, a mark of distinction is the endurance of one's scientific findings and the influence that they have decades later. Bill's early work helped define the role of the cholinergic nervous system in pain, an area that remains under intensive study. Bill's early work on endogenous opioids was important to our understanding of morphine's analgesic actions. However, his contributions also extended to other disease states. For example, Bill led a research team that discovered naltrexone was beneficial in treating SIDS. And of course, Bill's pioneering work was instrumental in laying the groundwork for the discovery of the endogenous cannabinoid system, a topic that is dear to me. He and his colleagues demonstrated enormous plasticity in this system which has become a major strategy for exploring the physiological role of endogenous cannabinoids. His early work on cannabinoid structure-activity relationships paved the way for proposing cannabinoid receptors and their ultimate discovery. Those early studies are still have a profound impact on cannabinoid research.

As for leadership qualities, Bill has never shied from a challenge. He has served as president of the American Society for Pharmacology and Experimental Therapeutics, Federation of American Societies and Experimental Biology, and the College. He served as Vice President for Research for Virginia Commonwealth University maintaining an active research laboratory. The list goes on.

Lastly, Bill has always been an individual who has wanted to help others. He has served as mentor to many students. As director of a National Institute on Drug Abuse training grant for more than 25 years, he has influenced the careers of more than 100 trainees. He has also been a mentor and positive role model for young faculty members, a great asset for the Department of Pharmacology and Toxicology at Virginia Commonwealth University.

In closing, I think it is most fitting to say that the most important thing to Bill is his family. I feel as though we are part of his extended family. I am honored to introduce the Nathan B. Eddy Memorial Recipient for the year 2000, William L. Dewey.

NATHAN B. EDDY MEMORIAL AWARD LECTURE

W. L. Dewey

Virginia Commonwealth University, Richmond, VA

CANNABINOID AND OPIOID RESEARCH AND ADMINISTRATION

Thank you boss. It is really something when your boss will say things as nice as that about you. I sincerely thank Drs. Horace Loh, Billy Martin and Lou Harris for initiating the nomination and for doing the hard work necessary to get all the material submitted. I also thank Drs. Marty Adler and Bob Schuster for taking time from their busy schedules to write letters of support. Thank you all and thanks to the members of the award committee for selecting me for this very prestigious award. I am very honored and humbled by this award. I can remember being so pleased when I was elected to the Board of Directors of CPDD quite a few years ago. It was a privilege and an honor to be on the board. That feeling was magnified a few years latter when I was elected to chair the board and now to be the recipient of the Nathan B. Eddy award is far beyond belief. It is most important to me to say a special thank you to my wife, Pat, who is here today and as many of you know her very important commitments usually prohibit her from attending meetings with me. She and our wonderful children made it possible for her to be here and that is most special. Certainly working hard so that Pat could be here today is only the last step in a progression of sacrifices by her and the children that allowed me to spend so much time and energy on my career throughout the years. In our family as I expect it is in most, all members pull together over many years and all contribute so much in so many ways to anything that is accomplished. Therefore it is on their behalf that I accept this award and we all thank you very much.

Like all of you in this profession, there is a second family, the people that we have the privilege to work with in this very exciting and fulfilling profession. As with my personal family I also have been very blessed here as well! The faculty at our institution working in the field of drug abuse are a marvelous group of scientists and even more importantly, they are a phenomenal group of human beings who make going to work a real pleasure. . The number has grown to more than 30 and there is an appropriate spread of experience from some of us who have been working together for more than 30 years to some newly appointed stars of the future who joined us recently. I started to work for Lou Harris 40 years ago this month at the Sterling Winthrop Research Institute. In every respect we have remained colleagues, friends and family. If it were not for Lou's encouragement, input and guidance throughout these four decades there is absolutely no way that I would be standing here today. I had the privilege to serve as the graduate faculty advisor for two of my current faculty colleagues, Drs.. Billy Martin and Sandy Welch. I am very proud of these two former students and have enjoyed long term collaborations with both of them throughout their careers. A vast majority of the work that I will summarize has been carried out with these colleagues. Long term collaborations with Mario Aceto, Bob Balster, Everette May, Ed Myer, John Rosecrans, Sid Schnoll and many others have been both enjoyable and very fruitful. I look forward to setting up similar collaborations with the newer faculty who have joined our group. Obviously, the work that has been published from our laboratory has been the result of the efforts of the 15 doctoral students, 30 post-doctoral fellows and visiting scientists and many hard working technicians who have provided excellent ideas and technical ability to our objectives. I make special note of the excellent leadership and cooperation of Drs. Sandy Welch and Forrest Smith who have made major and beyond the call of duty commitment to our laboratory during the past thirteen years while administrative responsibilities competed with my concentration on laboratory responsibilities. These two excellent scientists and leaders kept the laboratory moving in the right direction during these times of conflict of commitment.

1. CANNABINOIDS

Our work on the cannabinoids started in the 1960s, while we were at the University of North Carolina. Delta-9 THC had been identified as the major active ingredient in marihuana and its structure had been determined. We concentrated on elucidating the pharmacological profile of this interesting compound and a number of structurally related analogues. We and others demonstrated that the CNS depression caused by the cannabinoids differed from that produced by barbiturates, tranquilizers and other known depressants in that the cannabinoid induced depression had a stimulatory component not seen with these other drugs. Decreased spontaneous activity decreased body temperature; catalepsy, ataxia and general depression were accompanied by a pro-convulsant type CNS stimulation. Cannabinoids were also found to decrease normal and hypertensive blood pressure. The effect of delta-9 THC on

the overt behavior of dogs was unique and was characterized by immobility accompanied by swaying in both a forward and backward as well as side to side directions. A sharp noise would bring the dog out of the immobility state for a short period of time followed by a resumption of the immobility. These effects were dose responsive and their uniqueness allowed this test to become one of the standard laboratory procedures for quantitating cannabinoid activity of unknown compounds.

Further experimentation by other laboratories and ours demonstrated that cannabinoids had a very large variety of cellular and systemic effects on many organisms. At least some of these effects suggested that this series of compounds would have therapeutic potential. However the myriad of effects were observed at very close to the same dose so that specificity of effect, which is needed in therapeutics could not be achieved. These observations led us to embark on a long-term study of structure activity relationships to try and elucidate the portion of the molecule that was responsible for each specific effect. This would allow us to develop compounds with less diverse activities and greater potential for therapeutic usage. Dr. Raj Razdan and his colleagues have provided exceptional expertise in the chemistry of these compounds and the collaboration between our groups goes on to this day. One of the early accomplishments of this collaboration was the identification of heterocyclic compounds with cannabinoid activity. Delta-9 THC and the other naturally occurring cannabinoids are somewhat unique for compounds with such pronounced effects on the brain since they contain only carbon, oxygen and hydrogen atoms. We reported in the early seventies the cannabinoid activity of compounds with nitrogen and sulfur in the molecule that provided the potential for enormous expansion of compounds that should be investigated for specific therapeutic potential in this class.

A second important finding at this time was our report on the identification of a water-soluble cannabinoid. Delta-9 THC and the other cannabinoids described up to that point were all water insoluble and required the use of one or more vehicle which often had activity of their own to be used for pharmacological investigations. The discovery of a water-soluble cannabinoid greatly increased the therapeutic potential for this series of compounds. The similarity between the history of the opioids and the cannabinoids in this regard did not go unnoticed. Prior to the discovery of heroin the first water soluble opioid, the plant material was smoked. Similarly, marihuana is generally smoked and the potential that a water-soluble cannabinoid might increase the incidence and potential danger of abuse of this substance could not be eliminated. Another very interesting compound developed from this collaborative effort was abnormal cannabidiol, a cannabinoid that we found to have little if any effect on the central nervous system but a significant effect on lowering blood pressure. Recent work with this compound, by our colleague Dr. George Kunos and his collaborators has led to their hypothesis of a new class of cannabinoid receptor in the periphery. . A final series of compounds studied as part of this effort were the hexahydrocannabinols that were synthesized by Drs. Everette May and Ray Wilson. We found these compounds, as opposed to other cannabinoids, to be as potent as morphine in antinociceptive tests, to have similar effects on brain chemistry and both effects to be blocked by the narcotic antagonists.

One of the more interesting pharmacological properties of the cannabinoids is the very pronounced tolerance that develops to many of their effects. As with most drugs, tolerance does not develop to all the effects of the cannabinoids. Cross-tolerance occurs among cannabinoids but not between cannabinoids and drugs from other classes. Our laboratory working with the laboratory of Dr. Don McMillan carried out a number of experiments designed to determine if the tolerance to delta-9 THC was due to an alteration in the metabolism of the drug. We reported that pigeons who were highly tolerant to the behavioral effects of delta-9 THC had the same blood level of the parent compound and its metabolites as drug naïve birds given the same dose of drug. This indicated to us that the tolerance was not a metabolic tolerance. Next, we found that the concentration of delta-9 THC in four brain areas was the same in tolerant and non-tolerant pigeons, thereby demonstrating that the tolerance was not due to alterations in distribution. Further we found that the daily administration of radiolabeled deltra-9THC resulted in the accumulation of radioactivity in the brain of pigeons which demonstrated that the levels of drug accumulate in the brain on repeated administration. These results were confirmed in rodents where again we found accumulation of drug following chronic administration. Similarly, Dr. Billy Martin found while working on his doctoral dissertation in our laboratory that the plasma levels of radioactivity following the injection of radiolabeled delta-9 THC were not different in tolerant versus non-tolerant dogs. He also found that in only one of twenty areas of the brain studied was there significantly less radioactivity in tolerant than in drug naïve dogs. Fractionation of brain tissue into sub-cellular fractions showed that the distribution of radioactivity was the same in each fraction other than in the synaptic vesicle fraction in tolerant versus non-tolerant dogs. The synaptic vesicle fraction had 40% less radioactivity in the tolerant versus the non-tolerant dogs suggesting that the tolerance to the behavioral effects of delta-9 THC is a pharmacodynamic tolerance at the sub-cellular level of the brain.

II. OPIOIDS

Our initial work with the opioids was directed towards the elucidation of the role of various neurotransmitters in the mechanism of action of this interesting class of drugs. The majority of our work in the early years had to do with the interaction of central cholinergic systems in the action of morphine. Many other laboratories and ours have studied the effects of noradrenergic, dopaminergic, serotonergic and for us especially the cholinergic neurotransmitter systems on opiate analgesia. Although it is clearly an oversimplification, this work can be summarized as follows: treatments or drugs that increase brain tone or turnover rate of a particular transmitter increases the effects of the opiates. Conversely, treatments or drugs that decrease tone or the turnover rate of the transmitter decrease the effects of the opiate. For instance, Alan Bloom working in our laboratory showed that opiates increase brain catecholamine synthesis at the dose and the time they produce antinociception. A direct correlation was observed between the antinociceptive effect of the opiates and an increase in brain catecholamine synthesis and the narcotic antagonists blocked both of these effects. This type of work was carried out in many laboratories and the general conclusions were as described above.

One of our initial studies into the potential role of cholinergic systems in the action of narcotic analgesics was a comparison of the antinociceptive potency of the muscarinic agent, oxotremorine, the cholinesterase inhibitor, physostigmine and morphine in the mouse tail-flick test. Oxotremorine was 100 times more potent and physostigmine was equally potent to morphine. Only the isomers of many cholinergic compounds that produced cholinergic responses were active as antinociceptive agents. The antinociceptive effects were blocked by atropine but not atropine methyl nitrate showing that the effects of these cholinergic agents were in the central nervous system. The antinociceptive but not the cholinergic responses of these drugs was blocked by the narcotic antagonist, naloxone. The cholinergic compounds differed from morphine in that there were no opiate-like withdrawal signs following the cessation of chronic treatment with these compounds. These interesting findings with the peripheral administration of cholinergic agents caused us to investigate the potential antinociceptive effect of acetycholine itself, which had to be injected directly into the brain. Acetylcholine was found to have and ED-50 in the tail flick test of 7.3ug, in the phenylquinone writhing test of 5.1 ug and in the acetylcholine writhing test of 4.2ug. The antinociceptive effect of acetylcholine was blocked by a series of narcotic antagonists with the same order of potency that they block the antinociceptive effects of narcotics. The antinociceptive effects of acetylcholine were potentiated by the cholinesterase inhibitor neostigmine and were inhibited by atropine but not atropine methyl nitrate or the nicotinic antagonist, mecamylamine.

In summary, there were a number of similarities between the antinociceptive effects of cholinergic drugs, including acetylcholine itself and the narcotic analgesics such as morphine. Both classes of drugs produce antinociception to which marked tolerance develops, there effects are at least additive and often synergistic, they are both potentiated by cholinesterase inhibitors and their effects are blocked by the narcotic antagonists. In further studies some time later we showed that the antinociceptive effects of both classes of drugs are decreased by calcium, potentiated by calcium chelators and decreased further by calcium ionophores. These data and direct measurement studies have shown that both classes of drugs release endogenous opioids from brain tissue and this release is correlated to dose and time of the antinociceptive effects of the drugs. In spite of these many similarities in the effects of these two classes of drugs that produce antinociception, there are a few very interesting ways in which they differ. The effects of both are blocked by the narcotic antagonists but it has been known for a long time that the minus isomer of the antagonists are active versus the narcotic analgesics and we found that the plus isomer inhibits the antinociception produced by the cholinergic agents. A second difference between the effects of the antagonists in blocking the two types of drugs is that the blockade is competitive for the opiates and non-competitive for the cholinergic agents. These differences are particularly interesting since the order of potency for a number of the antagonists are the same verses both series of drugs. One other difference between the antinociception produced by the cholinergic drugs verses the narcotic agents is that alterations in brain catecholamine or serotonin systems did not alter the effects of the cholinergic agents as they do the opiates.

We have used the rodent tail-flick test as one of our standard procedures for quantitating antinociception. In experiments carried out in the late sixties, we showed that the activity of morphine, oxotremorine and physostigmine were reduced markedly in mice with severed spinal cords. About ten years later, Dr. T. C. Fu from Taiwan joined our laboratory and carried out a large series of elegant experiments in which he investigated this phenomena in more detail. He showed that when the neural component of the spinal cord was severed very carefully leaving the humoral component in tact there was no decrease in the activity of morphine in the tail-flick procedure. When the

humoral component was interrupted without damaging the neural component there was a marked decrease in the effect of morphine in this procedure. These results suggest that morphine releases an endogenous substance from the brain that is transported down the spinal canal in the cerebrospinal fluid to inhibit the spinal reflex. In order for this to be plausible we investigated the rate of passage of endogenous peptides in the cerebrospinal fluid to determine whether they could be transported from the brain to the sacral region of the spine at the time of peak activity of morphine in this procedure. Dr. Agneta Ohlson a visiting scientist from Sweden injected radiolabelled opioid peptides including beta-endorphin intraventricularly and radioactivity was quntitated in the lumbar and sacral regions of the cord ten minutes later. High concentrations of radioactivity correlated with the time of peak antinociceptive activity of morphine. These experiments did not refute the hypothesis. To test this hypothesis further, we quantitated the opioid activity of cerebrospinal fluid following the injection of a radiolabelled sample of morphine perenterally and found that the opioid activity of the csf was greater than could be accounted for by the radioactivity found in the cerebrospinal fluid. We concluded that the opioid activity in the csf in these experiments, which exceeded that from the total of morphine and its radiolabelled metabolites, was due to the release of endogenous opioids.

This series of experiments led us to make the following hypothesis as to the mechanism of action of morphine in producing analgesia and a hypothesis on the mechanism for the development of tolerance to opiates. We propose that morphine causes the release of endogenous opioids from synaptic vesicles into the synapse, which then interact with opioid receptors at the post-synaptic site. Considerable evidence has been generated to support this type of mechanism for the action of amphetamine and its ability to release dopamine from pre-synaptic vesicles to then interact with dopamine receptors. It is further proposed that during chronic treatment with morphine the stores of endogenous opioids are depleted and the reaction to morphine therefore is decreased. Larger doses of morphine are needed to overcome this tolerance and it is proposed that these larger doses allow the morphine to interact directly with the post-synaptic opioid receptor which has less affinity for morphine than for the endogenous opioid peptides.

Considerable evidence from our and other laboratories supports the role of the release of endogenous opioids. Many laboratories have shown that acupuncture which produces antinociception in laboratory animals as well as in man can be inhibited by the narcotic antagonist, naloxone. These results suggest that acupuncture releases an endogenous substance which itself is blocked by the antagonist. It has been reported that acupuncture causes an increase in opioid peptides in cerebrospinal fluid. One of our contributions to this literature was to show that acupuncture produced antinociception in hypo-physectomized mice and this effect was blocked by naloxone. These experiments show that the endogenous substances were being released by the brain and not the pituitary, which had been hypothesized. It has been reported by many investigators that there is an increase in the release of endogenous opioid peptides following stress induced antinociception and that surgery, strenuous exercise, physical stress, emotional stress and electroconvulsive treatment all release opioid peptides. We carried out a large series of experiments with Drs. Eastman and Pierce, two exercise physiologists from The University of Richmond in which we showed that there was a direct correlation between plasma level of beta-endorphin and subjective feelings of euphoria in new and experienced scuba divers. They had observed that first time scuba divers experienced a "high" after their dive. This was not observed when the divers had gained considerable experience with the sport. We found in double blind studies that the plasma levels of beta-endorphin was significantly elevated in the naïve divers but not in those with considerable experience with the sport. These experienced divers did not report a "high" in these studies which had been reported by all the naïve divers.

Dr. Edwin Myer a pediatric neurologist in our medical center, knew that we were studying the role of endogenous opioid peptides in the mechanism of action of morphine and initiated a long term collaboration directed toward determining the potential role of endogenous opioid systems in diseases characterized by centrally induced respiratory depression. Dr. Myer had been treating a child who had respiratory depression through out the five years of his life and the accepted treatments of the time were not adequate. Theophyline, anticonvulsants, atropine and a number of other drug treatments were tried with less that total success. The infants were kept on a monitor and resuscitation was often required. The child was given naltrexone and a significant reduction in the respiratory depression was observed. We discussed the possibility that an aberration in the endogenous opioid system might be involved in various disease states characterized by centrally induced respiratory depression. Sudden infant death syndrome (SIDS) is a disease often characterized by centrally induced respiratory depression that causes approximately 6-7,000 deaths in the U.S. each year. Infants are most susceptible during the first year of life and it has been shown that the disease has a genetic component and that there are a number of conditions that increase the risk of SIDS. These include a twin or a sibling of a child with SIDS, a child who has had an interrupted episode of

respiratory depression, premature birth, graduates of intensive care nurseries and children with abnormal breathing or cardiac patterns.

In our first study we investigated the levels of beta-endorphin in the plasma and the cerebrospinal fluid in a group of infants at a high risk for SIDS and a group who were not considered to be at a risk for SIDS. We hypothesized that those at a high risk for SIDS would have an elevated level of this endogenous opioid peptide in their blood and we would be able to identify those who needed to be placed on a monitor. There was no significant difference in the levels of beta-endorphin in the blood of the two groups. We did find an elevated level of the opioid peptide in the CSF of the infants considered being at a high risk for SIDS. We greatly expanded the number of infants in these groups in subsequent experiments and found that the mean level of beta-endorphin in the CSF of high-risk infants was approximately three times the average level for non-risk infants. There was no overlap in the data for the first eighty patients studied. That is, the level in the control was always lower than any number observed in the high-risk group. A representative case history: a six-month-old baby was admitted because of recurring life-threatening apnea requiring intubation and ventilation. Etiology of the respiratory depression was unknown and the beta-endorphin level in the cerebrospinal fluid was 70 pg./dl, which is more than double the level in controls. Oral naltrexone was initiated at a dose of l mg/kg and respiration became normal. Naltrexone was used in this fashion to treat a number of infants who had been on monitors for centrally induced respiratory depression and the monitors did not go off in infants treated with this drug. We attempted to set up a double blind controlled study to determine the efficaciousness of naltrexone but were unsuccessful. Obviously it is necessary to inform the parents that half of the patients would get the standard treatment while the other half would be given naltrexone. When the parents asked whether they could not participated in the study and get naltrexone for their infant all parents refused to participate in the study. In subsequent experiments we showed that the levels of other opioid peptides such as the enkephalins were also altered in the CSF of infants with centrally induced respiratory depression. Naltrexone was found to be useful in the treatment of other diseases where centrally induced apnea was a symptom. These diseases included Rett Syndrome, some convulsive disorders and autism. The results with naltrexone were not as impressive in these diseases as it was in SIDS and more work is needed to determine if the reversal of centrally induced respiratory depression by naltrexone in these diseases is a useful treatment of this system.

We and others and have shown that morphine decreases the level of intracellular calcium in the brain. The intraventricular injection of calcium blocks the antinociceptive effect of morphine and the antinociceptive effects of the cholinergic agents. However, when calcium is injected into the spinal cord it produces antinociception, which was blocked by naloxone and by the blockers of calcium gated potassium channels. We have demonstrated that the intrathecal injection of calcium into the spinal cord caused the release of endogenous opioids in that region. We hypothesize, therefore, that the mechanism of the antinociceptive effect of calcium is working through these released endogenous opioid peptides. As mentioned above, the acute injection of morphine to laboratory animals causes a decrease in the intracellular concentration of calcium, it has been show by many groups including ours that chronic treatment with opiates produces an increase in the intracellular concentration of calcium. The increase in intracellular calcium was accompanied by an opening of calcium channels in the cellular membrane and a release of calcium from intracellular pools. This apparent dichotomy between the effects of acute and chronic treatments of opiates on intracellular processes is not limited to calcium. Acute treatment with morphine causes a decrease in cyclic AMP levels and a decrease in protein kinase activity following acute treatment and an increase in level and activity respectively, following chronic treatment with morphine. To some extent acute treatment with opiates slows or decreases the activity of the cell and chronic treatment increases the activity of these intracellular components in a manner to compensate for the acute effects.

The observation that protein kinase C was increased in the brain and spinal cord following chronic treatment with morphine caused us to hypothesize that inhibitors of this enzyme would be able to reverse the tolerance that develops to morphine. We investigated the effects of blocking each step of the phosphatidylinositol cascade on the expression of morphine tolerance. In this series of experiments we implanted two groups of mice with 75mg morphine pellets and two groups with placebo pellets. Seventy-two hours later, at the time of peak tolerance, we injected the inhibitor to one group of mice with the morphine pellet and one group with the placebo pellet. The other group of mice with each type of pellet implant was given vehicle injections. A dose response curve for morphine was determined in all four groups of mice at the appropriate time thereafter. This design allowed us to compare the antinociceptive effects of acute morphine, with those of chronic morphine and those of chronic morphine plus inhibitor. In each experiment we also tested the effects of the inhibitor itself in placebo pelleted animals. In the initial studies, we found that both protein kinase A and protein kinase C inhibitors reversed the tolerance to morphine when tested 30 minutes after the intraventricular injection of the inhibitor. The protein kinase A inhibitor

Kt-5720 was not active when tested at 6 or 24hrs after injection. The protein kinase C inhibitors Go 7874, and Sangivamycin were active at 4, 8 and 24hrs after injection. The protein kinase inhibitors did not alter the ED-50 of morphine in mice implanted with placebo pellets but they both significantly reversed the tolerance that developed to the 72hr. implantation of morphine pellet.

It is our hypothesis and we believe that the field has accumulated a compelling case that there are multiple converging cellular events that contribute to opiate tolerance. The manipulation of these phenomena with selective tools allows us to inhibit specific steps in important pathways and gain a better understanding of the critical biochemical systems involved and the scientific basis of tolerance. The ability to reverse morphine tolerance has important implications, which should lead to a better understanding of the mechanisms involved in its development. In addition to the value of this research to the basic understanding of tolerance, we expect these studies to point in directions of clear clinical potential. The ability to reverse opiate tolerance in the clinic should lead to much improved methods of treatment of chronic pain.

III. ADMINISTRATIVE RESPONSIBILITIES

It is my opinion, that contributing to the administrative aspects of the discipline, the specific unit such as the department, the overall organizational unit such as the university, company or governmental branch of our employers and in fact to all society is a serious responsibility for all of us in science. It is absolutely essential that scientists be involved in decision making at all levels of administration of every entity that affects our ability to do science. Certainly this includes governmental decisions at all levels on the funding of science. This should be thought of as a federal responsibility for the most part but not entirely. Decisions concerning the distribution of resources including funds within the institution needs input from experienced scientists. It is essential that productive scientists play important functional roles in the activity of their professional organizations in these efforts. It is these groups of scholars in a particular discipline working together on the specific issue that can provide important input that can not be provided by individuals outside the specific area of research. The generation of knowledge, a most satisfying and rewarding accomplishment, is the primary function of academia, industry and the scientific branches of government. It is most difficult to understand the importance of each of the complex and even the everyday mundane aspects of running a productive laboratory unless you have participated in one and actually ran one yourself.

I may have such strong opinions on this subject because it is here where I have spent the majority of my effort over the past 13 years. Thanks to the exceptional work and cooperation of Drs. Sandi Welch and Forrest Smith and the other members of our laboratories we were able to maintain productivity and keep my research passion growing. Nonetheless, I was being paid to be a university administrator with major responsibility for research and graduate education. It was in this position that my earlier opinion that it was essential for a scientist to have input in the decision processes on matters that affected the ability of scholars to accomplish their mission was confirmed on a regular basis. The appropriate demand for excellence in teaching at both the undergraduate and graduate levels, as well as the continually increasing fiscal demands, could cause scholarship to become less than a high priority in the decision making process unless someone with a committed interest represents this area in the deliberations. Unfortunately, even that isn't always enough.

Clearly, as indicated above there are an endless number of ways that a scientist can have continual impact at an administrative level but there are also a number of specific initiatives that one can undertake which are very significant to ones own discipline. As I have discussed above, the contributions of the faculty in the field of drug abuse at our institution have been the standard by which all others are judged. This certainly was an important contributor to our success in two initiatives, which further benefited our group of scientists. The Commonwealth of Virginia initiated a support mechanism in 1988 to encourage intra-disciplinary work in areas of expertise within an institution. The Commonwealth Center on Drug Abuse was the one project submitted for funding from our institution at that time. This initiative provided more than 1.5 million dollars in support and an additional tenure eligible position. It was through this mechanism that we were able to hire Dr. Mary Abood as the first molecular scientist in our group. Awards were made for a five-year period without the possibility for extension or renewal. Later our university decided to support inter-disciplinary groups both fiscally and administratively. Dr. Bob Balster is the Director of the Drug and Alcohol Studies Institute, which was initiated in 1998 with a ten-year commitment including significant financial and administrative support to help maintain and increase drug and alcohol research. The recent, significant expansion of the drug abuse faculty at our institution would not have been possible without this support mechanism. These two programs have provided significant internal support to supplement the funding

that this group of scholars has been able to generate from external sources. The reason this was accomplished was in no small way due to the active involvement of many faculty in this research group in the administrative and decision making processes of the institution. Clearly this type of involvement pays off within the institution and at all levels of government.

There are many issues that have a direct effect on the ability of the scientists to carry out their work and these issues require the active participation of the scientists themselves in as many aspects of the decision making process as possible. At least two of these areas demanded a significant portion of my time and energy. They are funding of biomedical research and the protection of the need to carryout humane biomedical research on laboratory animals. It is obvious that both of these issues are essential to pre-clinical work in the drug abuse field. University administrators and scholarly society officers must take a leading role in securing adequate funding for research from all levels of government and to communicate to them the tremendous contributions of basic biomedical research to the quality and quantity of life which man now enjoys. One aspect of this leading role is to encourage all scientists to become active participants in the process. It is no doubt that the greater the participation and the more organized the effort, the better the results. It does make a difference to appear before local, state and federal governmental bodies to explain the benefits of biomedical research to their constituents, their families and themselves.

In summary, ladies and gentlemen I hope I have made it clear that this has been a real joy ride. Doing research and discovering new knowledge is an amazingly rewarding and enjoyable way to earn a living. I am extremely grateful to all my colleagues who have contributed so much to the research that I have described and to making each day such fun. Faculty, visiting scientists and postdoctoral fellows, students, laboratory and office colleagues have all made significant contributions to any progress that has been made. It has been a family working hard and together that has made the past decades pass so fast. I think the strongest drive to do research is the gratification that it brings. Research is difficult and very demanding and we all do it for just one reason: we really enjoy it. Most importantly, I want to thank my own family, Pat our six children and the rest of our immediate family who always recognized the absolute necessity for them to be active contributors in these efforts by always carrying out more than their share of the burdens and activities of the family so as not to interfere with my work in the university, in scholarly societies, or other aspects of the profession. This clearly has been a team effort and it is on behalf of my family, without whom nothing would be important, that I humbly and gratefully accept this award.

SYMPOSIUM II

ABUSE POTENTIAL ASSESSMENT OF GLUTAMATE ANTAGONISTS

R. L. Balster and R. S. Mansbach, Chairpersons

Speakers: R. L. Balster, W. Danysz, K. M. Nicholson, H. de Wit, M. Klein, and R. S. Mansbach

OVERVIEW

Drugs that antagonize excitatory amino acid neurotransmission have many potential clinical uses. These include use for treatment of neurodegenerative diseases, neurotrauma and stroke, convulsions, various psychiatric conditions and pain. These drugs have also been shown to modify the development of tolerance and dependence to drugs of abuse and have been proposed as drug abuse treatments. One of the best-known glutamate antagonists is phencyclidine (PCP) and there is concern that other drugs of this class will have PCP-like side effects and abuse liability. Assessment of the abuse potential of glutamate antagonists provides some special challenges to the drug abuse research field.

WHY MAY ABUSE POTENTIAL ASSESSMENT OF GLUTAMATE ANTAGONISTS BE NEEDED?

R. L. Balster

Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA

Various types of glutamate antagonists are currently used or being developed as medications for a wide variety of uses. For new compounds especially it will probably be necessary to conduct preclinical and clinical studies of their abuse potential. There are three principal reasons for this. 1) The best characterized glutamate (NMDA) antagonists, phencyclidine and ketamine, have known abuse potential and are controlled substances in the United States. 2) The pharmacological profile, as well as some of the indications, for glutamate antagonists are similar in many ways to those of GABA agonists, a diverse group of compounds some of which are abused. Whether glutamate antagonists have abuse potential of the CNS depressant type should be considered. 3) Many glutamate antagonists that have been tested in human subjects produce psychotomimetic-like acute effects at high doses. These acute effects have not been well studied in humans, but do appear to be different from the effects of classical hallucinogens from the phenethylamine and indolamines classes. Whether or not glutamate antagonists may have abuse potential as "hallucinogen-like" drugs has not been clearly established.

POTENTIAL THERAPEUTIC APPLICATIONS OF NMDA RECEPTOR ANTAGONISTS – PRECLINICAL AND CLINICAL STUDIES

W. Danysz, C.G. Parsons, and G. Quack

Department of Pharmacological Research, Merz+Co, Frankfurt /M, Germany

Glutamate is involved in virtually all functions of the CNS. This, on one hand, gives the basis for using drugs which modify glutamatergic neurotransmission as therapeutic interventions in many brain dysfunctions, but, on the other hand, also for potential side-effects. Hence, in recent years the challenge has been to develop new agents with acceptable therapeutic tolerability. It should be stressed that there are already agents in clinical use, such as memantine, which provides the proof of concept, i.e. the question is not whether NMDA antagonists can be used in therapy, but rather what features such compounds should have. Hence, it seems at present that there is a consensus that competitive NMDA receptor antagonists have low chances of finding therapeutic applications, in contrast to agents acting at the glycine$_B$ site, or channel blockers. At present there are at least 7 glycine$_B$ antagonists (e.g. ACEA 1021, GV-150526, GV-196771A, ZD-9379, MRZ 2/576) and over 10 NMDA channel blockers (e.g.

Cerestat, Remacemide, ARL-15896AR, HU-211, ACDI, CNS-5161, MRZ 2/579) under development. Additionally, substances showing satisfactory selectivity for certain NMDA receptor subtypes seem to have a favourable profile. An example could be eliprodil, CP-101606 or Ro-25-6981 being selective antagonists of NMDA receptors containing NR2B subunit. The present presentation is an attempt to critically review preclinical and scarce clinical experience in the development of new NMDA receptor antagonists according to the following scheme: rationale, preclinical findings in animal models and finally clinical experience if available. Special attention will be devoted to the difference between agents acting at various recognition sites of the NMDA receptor. The present experience with NMDA receptor antagonists could be summarized as follows:

Disorder	Rationale	Animal models	Clinical evidence
Stroke	+	+	-
TBI	+	+ /- SC	-/?
Huntington's - neuroprotection	+	+/-	?
ALS – neuroprotection NMDA/AMPA r. antagonists	-/+	-/+	-/+
Parkinson's disease – protection	+	+/?	+/?
Neurodegenerative dementia – protection	+	+	?
Parkinson's disease – symptoms	+	+	+/?
Parkinson's disease - dyskinesias	+	+	+
Neurodegenerative dementia - symptoms	-/+	-/+	+
Epilepsy – complex partial seizures	+	+/-	-
Depression	+/-	+	+/?
Alcohol abuse	+	+	+
Opioid abuse	+	+	+
Morphine Tolerance	+	+	+/-
Chronic pain	+	+	+/-

+ - positive evidence; - negative evidence; ? – lack of data; SC – spinal cord injury; TBI – traumatic brain injury

PRECLINICAL METHODS FOR ASSESSING ABUSE POTENTIAL OF NMDA ANTAGONISTS

K. L. Nicholson and R. L. Balster

Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA

Preclinical assessment of novel NMDA antagonists for abuse liability incorporates three avenues of investigation: determination of pharmacological equivalence to phencyclidine (PCP) or other potent NMDA channel blockers; evaluation of reinforcing properties; and testing for the development of tolerance and dependence. Novel antagonists are evaluated *in vitro* for similarity to PCP based on chemical structure, receptor binding profile and ability to antagonize NMDA/glutamate stimulated cellular events. *In vivo*, a variety of procedures are used to evaluate NMDA antagonists' unique combination of CNS depressant and psychomotor stimulant characteristics to help predict equivalence to PCP. The most selective and validated method for predicting equivalence to PCP-like drugs is testing for substitution utilizing drug discrimination procedures. Drug discrimination studies in animals are considered to be predictive of subjective effects in humans and therefore useful in abuse potential assessment. The reinforcing properties of NMDA antagonists have been assessed using self-administration procedures in rodents and monkeys. The results of these procedures have demonstrated a good correlation between drugs self-administered by animals and those abused by humans. While both tolerance and dependence have been shown to develop following chronic high dose administration of PCP in animal models, this has not been extensively studied for other glutamate antagonists. More research is needed to determine the utility of these procedures for predicting abuse potential.

Results of animal testing so far suggest that many channel blocking NMDA antagonists produce PCP-like discriminative stimulus and reinforcing properties and would be predicted to have PCP-like abuse liability. Competitive antagonists have both similarities and differences to PCP so may not be equivalent to potent channel

blockers in abuse potential. Glycine- and polyamine-site antagonists thus far appear to produce few PCP-like effects. Development of NMDA antagonists for therapeutic use is currently focused on the latter classes of drugs which would be predicted to have limited PCP-like side effects in humans. Interest also surrounds antagonists that demonstrate selectivity for NMDA receptors containing NR2B subunits and novel channel blockers whose unique binding profiles are purported to diminish PCP-like side effects. In conclusion, validated animal models are available which should be useful in predicting PCP-like side effects and abuse liability. Based on preclinical results to date, there is a good possibility that not all NMDA antagonists will have significant PCP-like abuse liability.

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METHODS FOR ASSESSING ABUSE POTENTIAL OF NMDA ANTAGONISTS IN HUMANS

H. de Wit

Department of Psychiatry, University of Chicago, Chicago, IL

NMDA antagonists are of interest in the substance abuse field both because certain drugs in this category may have potential for abuse themselves whereas others are under consideration in the treatment of substance abuse. Assessing the abuse potential of NMDA antagonists presents a number of challenges to the clinical researcher. First, this category of drugs represents a heterogeneous group of compounds with varying mechanisms of action, a wide range of behavioral and subjective effects and several different potential therapeutic applications. Second, many NMDA antagonists produce unique and unfamiliar sensations and subjective experiences (e.g., depersonalization or perceptual distortion) which are often difficult to describe and for which no standardized measures exist. Third, partly because of the heterogeneity of this class of drug and partly because of the limitations of the self-report instruments, there is no accepted and well-characterized drug to serve as a standard of comparison against which to test other drugs. Finally, the patterns of recreational use or abuse of drugs with hallucinogenic properties may differ fundamentally from use and abuse of other, more traditional drugs of abuse. For all of these reasons, it is unlikely that any single clinical protocol will be appropriate for assessing the abuse potential of all NMDA antagonists. Rather, abuse liability assessments of this class of drugs will probably need to be designed individually for each compound under consideration.

Two main sources of data can be examined to assess the abuse potential of NMDA antagonists. First, controlled laboratory studies can be conducted to assess the subjective effects of the drug, including measures of euphoria and ratings of drug liking. Second, preliminary information about abuse potential can be gathered from all phases of clinical trials during the drug development process. Several principles should be followed in conducting both types of studies, such as the inclusion of appropriate subject samples, use of a known, standard drug for comparison, a wide range of doses including supratherapeutic doses, and sensitive, well-validated self-report questionnaires. Care should be given to address the safety and ethical issues that arise in testing this unique class of drugs.

DISCUSSANT: FDA PERSPECTIVE

M. Klein

Food and Drug Administration, Rockville, MD

FDA/CDER, in assuring the safety and effectiveness of drugs, works collaboratively and cooperatively with industry, academia, and others to improve and expedite the drug development and review process. Specific regulations apply for expediting the development of drugs for debilitating and life-threatening conditions. Early identification of concerns in the development process is a primary goal. The issue of a drug's abuse potential needs to be addressed prior to submission of the new drug application. The *N*-Methyl-D-Aspartate (NMDA) receptor is an attractive target in drug development and has been widely described as potentially offering important new therapeutic applications, as neuroprotectants in stroke, head trauma, hemorrhage, and as a pharmacotherapy for drug abuse and addiction to decrease the frequency and severity of relapse. From the drug development viewpoint, there are many areas of concern and ongoing research for the NMDA antagonists that range from toxicology issues to concerns about the demonstration of clinical efficacy and safety. It has been widely stated that the therapeutic potential of NMDA antagonists may be limited because of psychotomimetic side effects and a history of abuse of certain prominent members of the class (specifically PCP and ketamine). In order to assess abuse as a possible

impediment to eventual development for important and new areas of treatment, FDA/CDER has continued to recommend comprehensive and thorough evaluation of the abuse potential of new drugs. Results from the abuse liability assessments provide important information that ensure proper product labeling, warnings, and appropriate use of the drug after marketing.

DISCUSSANT-INDUSTRY PERSPECTIVE

R. S. Mansbach

Department of Neuroscience, Pfizer Global Research and Development, Groton CT

Abuse potential and associated controlled scheduling represent one of many hurdles drug companies face in bringing new CNS therapies to market. With the identification of cellular mechanisms of action for all major classes of abused drugs, a heightened awareness exists that ligands developed to interact with novel targets may in some cases impact reward systems in the brain and therefore represent a risk for misuse or illicit diversion. The decision to prosecute discovery programs for compounds that may eventually be scheduled depends on several factors. The negative consequences of schedule control (in the form of decreased sales and increased liability) may be mitigated if there is a considerable unmet medical need, if all major approved medications are also scheduled, or if the setting in which the drug is administered decreases physicians' concerns over prescribing it (e.g., inpatient-only use). In contrast, entry barriers may be high if existing therapies are efficacious and do not suffer from major weaknesses. Moreover, some medications with reinforcing properties receive even more scrutiny and restriction internationally than in the U.S. In order to help manage the risk of eventual schedule control, it is of value to conduct market research with specialists and primary care providers, with the goal of understanding trade-offs between abuse liability and other attributes that may be highly valued in clinical practice. It is also of value to conduct early abuse liability studies in laboratory animals and healthy volunteers, particularly if the novelty of the molecular target makes it difficult to predict the drug's potential for producing reinforcement or physical dependence. A successful abuse liability plan requires a thorough understanding of the compound's pharmacological and behavioral properties, as well as close cooperation with regulatory agencies and outside scientific collaborators.

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SYMPOSIUM III

DRUG ADDICTION TREATMENT FOR WOMEN: DOES GENDER MATTER?

C. L. Wetherington and B. Tai, Chairpersons

National Institute on Drug Abuse, National Institutes of Health, Bethesda, MD

While progress has been made in developing efficacious behavioral therapies and pharmacotherapies for treating drug abuse and dependence, research on their differential effectiveness in men and women has received insufficient attention. In addition, little research attention has been paid to the development of gender-sensitive treatment approaches despite scientific evidence suggesting that drug abuse and dependence progress differently in males and females, and therefore may require different treatment approaches. There is a critical need to increase the attention to women and gender difference in clinical trials of behavioral therapies and pharmacotherapies and also in trials addressing the transportability of effective treatment to "real world" community-based settings. The purpose of this symposium was to discuss these issues and to provide information on gender differences in treatment research and the utility of gender-based research, and to consider recommendations that will aid in moving the research in this area forward.

GENDER DIFFERENCES IN THE BIOLOGICAL BASIS AND PROGRESSION TO DRUG ABUSE: IMPLICATIONS FOR TREATMENT

C. L. Wetherington

National Institute on Drug Abuse, National Institutes of Health, Bethesda, MD

As recently as a decade ago, the National Institute on Drug Abuse (NIDA) supported virtually no gender-based research and very little research on women. Most of the research on women was from a pregnancy perspective, and in particular, the concern over the possible adverse effects on offspring prenatally exposed to drugs. In the 1970's NIDA began funding research on the effects of prenatal exposure to heroin and marijuana. And in the late 1980's, with growing public concern over the possible effects of prenatal exposure to cocaine, NIDA launched a major initiative that funded twenty treatment demonstration grants targeting drug-abusing pregnant and postpartum women (the "Perinatal 20"). Subsequently, concerns emerged regarding the status of what was known about treatment of non-pregnant women and about women and other aspects of drug abuse, and how males and females differ in all aspects of drug abuse. To assess these issues and to identify research needs, in 1994 NIDA held a conference, "Drug Addiction Research and the Health of Women." From that conference, several broad areas of drug abuse research needs were apparent: the need to study all females, not just those of child-bearing age; the need to study gender differences; the need for basic research on females and gender differences; and, the need to study females and gender differences in all areas of drug abuse research. Since then, NIDA has actively engaged in a variety of efforts to fill these research gaps on women and gender differences. The drug abuse research field has responded to this need as evidenced by the number and breath of presentations on this topic at this year's CPDD meeting. NIDA is pleased to have developed for this year's CPDD a mini-program book on women and gender differences extracted from the CPDD program book. It contains nearly 130 program entries on the topic and represents over 20% of the CPDD presentations.

In virtually all areas of drug abuse research, sex differences are beginning to emerge. Among the areas are epidemiology, behavioral animal models, the menstrual cycle, and etiology and progression.

EPIDEMIOLOGY. In general, the U.S. population prevalence for both drug use and dependence is higher for males than for females. This could suggest that females are less vulnerable to drug abuse and dependence than males. Recent research, however, indicates an important reinterpretation of these population prevalences. Using 1993 NHSDA data, Van Etten *et al.* (1999), found that males are more likely than females to report that they have had an opportunity to use marijuana, cocaine, heroin, and hallucinogens, and that calculation of the use prevalence of these drugs for only individuals who report an opportunity to use indicates that the conditional probability is

equivalent for males and females. Similarly, Anthony *et al.*, 1994, using data from the National Comorbidity Survey, determined that when drug dependence prevalence for specific drugs is calculated only for those individuals who have used them, males and females are equally likely to become dependent upon cocaine, heroin, hallucinogens, tobacco, inhalants, and analgesics. Males, however, are more likely to become dependent on marijuana and alcohol given use, while females are more likely to become dependent on anxiolytics, sedatives, and hypnotics. Kandel *et al.* (1997), reported similar conditional probabilities of dependence using NHSDA data. Taken together, these epidemiologic data do not support the notion that males are more vulnerable than females to either drug use or dependence although males are more likely to have opportunities for use. These data suggest that if future trends show an increase in opportunities for females to use drugs, the prevalence of female use, dependence, and need for treatment also will increase

BEHAVIORAL ANIMAL MODELS. In behavioral animal models, sex differences are emerging along several lines of research. (1) Studies of the locomotor response to psychostimulant drugs in rodents have shown than females exhibit greater responsivity to cocaine (van Haaren, 1991, Haney *et al.*, 1994) and methamphetamine (Schlindler *et al.*, CPDD 2000) and that the responsivity is greater during the estrus phase of the estrus cycle (Becker *et al.*, 1989; Quinones-Janab *et al.*, 1999). (2) Compared to males, female rats have greater levels of self-administration of caffeine (Heppner *et al.*, 1985), cocaine (Matthews *et al.,* 1999; Morse *et al.*, 1993), morphine (Alexander *et al.*, 1978; Hill, 1978), fentanyl (Klein *et al.*, 1997), and alcohol (Hill, 1978; Lancaster & Spiegel, 1992). Interestingly, in humans, DATOS data indicate greater daily use of cocaine, heroin, and sedatives and barbiturates by females than by males (Wechsberg, 1998). (3) Sex differences in the reinforcing effectiveness of cocaine was reported by Roberts *et al.* (1989), who found considerably higher breakpoints on a progressive ratio schedule in female rats than in males, a finding consistent with human data (Haney *et al.,* 1998). And, Lynch and Carroll's (2000) report that female rats exhibit reinstatement of extinguished responding at a lower priming dose suggests greater sensitivity to cocaine's reinforcing effects in females. (4) Study of sex differences in the acquisition of drug-taking indicates that female rats acquire self-administration of both cocaine and heroin faster than males (Lynch & Carroll, 1999). (5) "Prevalence" of self-administration of cocaine appears greater in female than male rats, as measured by the percentage of rats that acquire self-administration; however, for heroin, sex differences do not occur (Lynch and Carroll 1999). (6) Finally, female rats exhibit greater reinstatement of extinguished responding than males (Lynch and Carroll 2000). Thus, behavioral data from animal models, like the epidemiologic data, do not suggest greater vulnerability or sensitivity to illicit drugs in males compared to females. The trend, in fact, is in the opposite direction.

MENSTRUAL CYCLE. Emerging research indicates that the phase of menstrual cycle is a determinant of the response to drugs and to cessation of use. Justice and de Wit (1999), found that in response to orally administered d-amphetamine, females report greater feeling of "high," greater euphoria, greater energy and intellectual efficiency, and greater liking and wanting of the drug during the follicular phase than during the luteal. Similarly, Evans *et al.,* (CPDD, 2000), found that for cocaine, females in the follicular phase, compared to the luteal phase, report more higher peak ratings of "high" and "good drug effect," and willingness to spend more money for cocaine. Perkins (2000), reported that females who attempt to quit smoking in the follicular phase have fewer withdrawal symptoms and less depressive symptomatology in the week after quitting than women who attempt to quit in the luteal phase. Aside from Perkins' study, the role of the menstrual cycle in the timing of treatment is largely unexplored.

ETIOLOGY & PROGRESSION. A variety of research findings indicate that the path to drug abuse is not the same for males and females. Females appear to use drugs for a shorter period of time than males before becoming dependent, a finding shown for cocaine (Griffin *et al.*, 1989), heroin (Hser, 1990), marijuana (Mezzich *et al.*, 1994), and alcohol (Blume, 1996). Depression is a greater predictor of drug use by male than female adolescents, while conduct disorder is a greater predictor of drug use by female than male adolescents (Costello *et al.*, 1999). Aggression during the first grade is a predictor of drug use in boys, but not in girls. Cigarette use is a greater predictor of progression to illegal drug use by girls than by boys (Kandel *et al.*, 1992, 1998). Smoking during pregnancy is associated with smoking by preadolescent female offspring, but not male (Kandel *et al.*, 1994; Weissman *et al.*, 1999). Several family factors are more predictive of drug use in females than males including maternal alcoholism (Boyd *et al.*, 1993), maternal drug abuse (Boyd *et al.*, 1993), low parental attachment (Ensminger *et al.*, 1982; Brook *et al.*, 1993), low parental monitoring (Krohn *et al.*, 1986), low parental concern (Murray *et al.,* 1983), unstructured home environment (Block *et al.,* 1988), and a dysfunctional family (Chatham *et al.,* 1999). Among drug abuse treatment populations, there are gender differences in psychiatric co-morbidity including whether drug abuse is primary or secondary, and thus the possibility of gender differences in the role of psychiatric disorders in the etiology and treatment of drug abuse and dependence. DATOS data indicate that female

adolescents in drug abuse treatment, compared to their male counterparts, are twice as likely to have experienced physical and/or sexual abuse (Rounds-Bryant *et al.,* 1998). And, finally, DATOS intake data (Wechsberg *et al.*, 1998) indicate that at treatment entry, compared to men, women are less likely to have graduated from high school, almost half as likely to be employed, more likely to report prior drug treatment, more likely to report physical or sexual abuse or both, and more likely to report depression, suicidal attempts and thoughts, health problems, and to be troubled over current emotional and psychological problems. Thus, compared to men at treatment entry, females have an overall lower level of functioning. The extent to which these characteristics play a role in the etiology of drug abuse and dependence or are a consequence requires further study. While it is clear that many of the predictors and correlates of drug use and dependence are not gender-neutral, their role in treatment outcome has been largely unexplored.

REFERENCES: Available from author upon request (National Institute on Drug Abuse, 6001 Executive Boulevard, Room 4282, MSC 9555, Bethesda, MD 20892-9555 or wetherington@nih.gov).

GENDER DIFFERENCES IN SMOKING CESSATION TREATMENT: IMPLICATIONS FROM THE LAB AND THE CLINIC

K. Perkins

Department of Psychiatry, University of Pittsburgh, Pittsburgh, PA

Compared with men, women may suffer greater relative risks of diseases due to smoking, including some cardiovascular diseases, lung cancer, and other pulmonary diseases. Women also suffer from smoking-related disorders not faced by men, such as reproductive complications. Therefore, improved treatment of smoking cessation in women could have a greater net benefit to public health than similar efforts aimed at male smokers. However, women tend to have less success than men in quitting smoking, particularly in formal clinical trials, although poorer outcome in women has been seen in studies of self-quitters. The difference between women and men in long-term smoking abstinence may be even greater in trials of nicotine replacement therapies (NRT), suggesting that women may benefit less than men do from NRT. By contrast, some non-NRT medications, such as clonidine and perhaps bupropion, mecamylamine, and naltrexone, may reverse the generally poorer outcome of women, producing rates of abstinence in women that are similar to those of men (Perkins, in press). These clinical observations suggest that nicotine may be less influential, and non-nicotine factors more influential, in promoting smoking behavior in women compared with men.

Research from the laboratory tends to support this notion. As with any drug dependence, smoking behavior may be seen as reinforced by pharmacological factors, primarily nicotine, and by non-pharmacological factors, primarily conditioned stimuli associated with nicotine intake from smoking ("cues"). Some non-nicotine effects of smoking, such as the sight and taste or smell of smoke, accompany each and every instance of nicotine intake from smoking. The pack/day smoker smokes over 7,000 cigarettes per year (and approximately 70,000 puffs), certainly the most frequent drug use behavior (and perhaps most frequent consummatory behavior of any kind) engaged in by humans. Such frequent behavior can produce highly learned associations between the non-nicotine stimuli and nicotine reinforcement. Compared with men, women's smoking may be influenced less by nicotine and more by non-pharmacological, conditioned factors. Manipulations of nicotine exposure per se tend to alter smoking behavior less in women than in men, while manipulations of non-nicotine stimuli associated with smoking tend to affect smoking behavior more in women than in men. For example, we have found that women self-administer nicotine by nasal spray less than do men (Perkins *et al.,* 1996), and women are less sensitive to the discriminative stimulus effects of nicotine (Perkins, 1999). Sex differences in nicotine sensitivity do not appear to be due to pharmacokinetic differences (i.e. lower or higher blood nicotine levels in women), which would be expected to produce uniform differences in responses to nicotine between men and women; only select sex differences in responding have been observed.

On the other hand, women tend to persist in smoking to a greater extent after pre-treatment with nicotine, while men compensate by reducing their smoking in dose-dependent fashion following nicotine pre-treatment. This finding suggests that women regulate nicotine exposure less tightly and/or smoke more for conditioned reinforcing effects. Removal of non-nicotine smoking-associated stimuli (e.g. lit cigarette "cue," smell and sight of smoke) without affecting access to nicotine per se reduces smoking reinforcement more in women than in men. Moreover, potential

conditioned reinforcers of smoking behavior are very numerous and can involve sensorimotor effects, timing of smoking behavior, and social factors (e.g. smoking with friends), each of which may be more influential of smoking in women than in men (see Perkins *et al.*, 1999 for a review). Reduced sensitivity to pharmacological factors and greater sensitivity to conditioned factors in women versus men may not be unique to tobacco smoking; some studies suggest a similar pattern of sex differences in sensitivity to cocaine use.

Specific mechanisms to explain sex differences in pharmacological versus non-pharmacological influences on smoking behavior deserve study. While sex hormone levels may be an obvious possibility, few studies have shown an effect of menstrual cycle phase on smoking reinforcement. Research with animal models may provide the experimental control necessary to clearly identify specific biological factors that may account for sex differences in responses to nicotine versus conditioned reinforcers. In addition, parallel findings between humans and non-human animals may indicate a profound biological difference between males and females that affects drug-taking behavior. On the other hand, if these sex differences in factors promoting drug use are specific to humans, "gender"-related factors, such as cultural expectations for women and social sex roles, may be responsible for the observed differences in smoking behavior.

These differences between men and women in the relative influence of nicotine and conditioned factors on smoking behavior have implications for development of new treatments for smoking cessation. Although women smokers clearly are nicotine-dependent and would benefit from NRT, non-NRT medications may deserve greater consideration for treating women. In addition, behavioral interventions to extinguish (or help smokers otherwise cope with) conditioned smoking stimuli would be expected to be particularly effective in women. Overall, greater attention to sex differences in clinical trial outcomes and to addressing concerns of women smokers may aid in the development of substantially improved smoking cessation interventions for women.

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GENDER DIFFERENCES IN COMORBIDITY: IMPLICATIONS FOR TREATMENT

K. T. Brady

Medical University of South Carolina, Department of Psychiatry, Charleston, SC

One area in which many important differences between male and female substance users have consistently been found is that of psychiatric comorbidity. A number of studies have demonstrated that anxiety and affective disorders are more common among women with alcohol, cocaine or opiate dependence as compared to men. Male substance users have been shown to have more antisocial personality disorder and more polysubstance dependence as compared to females (Brady *et al.*, 1993). One issue of particular importance to gender differences in psychiatric comorbidity is that of victimization and violence. Victimization, associated with PTSD as well as other psychiatric disorders, has been shown to be commonly associated with substance use disorders for women in particular. Both epidemiologic and studies of clinical samples demonstrate that for anxiety and affective disorders, the onset of psychiatric disorder precedes the onset of substance use more often in women than in men. These gender differences in order of onset of psychiatric and substance use disorders may have both etiologic significance and implications for gender-specific treatment. If "self-medication" is a more relevant construct for women as compared to men, treating psychiatric disorder may have a differential effect on treatment outcome in women. There has been little systematic research on gender-specific treatment, but the higher comorbidity with affective and anxiety disorders in women and, in particular, the difference in order of onset certainly suggests avenues for further

exploration. In a large, multisite treatment matching study of various approaches to relapse prevention in alcohol dependent individuals (Project MATCH, 1996), high scores on the psychiatric subscale of the ASI were predictive of a preferential response to cognitive behavioral therapy (CBT) as compared to 12-step or motivational enhancement therapies. Although gender differences in treatment response were not found in the overall data analysis, analysis of specific comorbid populations has revealed gender differences. In an analysis of the subset of individuals with social phobia in this study, Randall and colleagues (in press) demonstrated that women with social phobia had a more robust response to CBT than to other therapies. This was not true for women without social phobia or for men with or without social phobia suggesting an interaction between gender and psychiatric comorbidity. Finally, several studies have shown that pharmacotherapeutic treatment of psychiatric disorders in individuals with comorbid substance use disorder can improve substance-related outcomes. Cornelius and colleagues (1996) demonstrated that fluoxetine treatment of depressed alcoholics improved both alcoholism and depression. There has been no reported analysis of gender differences in pharmacotherapeutic treatment response for substance using populations with psychiatric comorbidity, but a number of studies have shown a more robust treatment response to SSRI's in women as compared to men. Exploration of both pharmacotherapeutic and psychotherapeutic treatment of psychiatric comorbidity in individuals with substance use disorders may prove to be an important area in investigations of gender-based differences in treatment response.

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BEHAVIORAL THERAPIES: DOES GENDER MATTER?

K. Moras

Department of Psychiatry, University of Pennsylvania, Philadelphia, PA

OVERVIEW

What answers do randomized treatment outcome studies provide to the question, Do women substance abusers need gender-specific behavioral therapies?

The term 'behavioral therapies' refers to verbal and behavioral interventions that are derived mainly from psychological theories and principles. Behavioral therapies that have been empirically tested for drug abuse include cognitive behavior therapy, contingency management techniques, interpersonal psychotherapy, relapse prevention, skills training, and 12-Step recovery programs. Several behavioral therapies used for drug abuse are described in Onken *et al.* (1993).

When considering whether women substance abusers might benefit from gender-specific therapies, heterogeneity must be considered. Like their male counterparts, female substance abusers can be sub-typed on several dimensions that could be treatment-relevant such as type and number of drugs abused, length and course of drug abuse, type of psychiatric comorbidity, type and degree of impairment in social roles, ethnicity and related values, parental status, and socioeconomic background. Some subtypes might benefit from gender-specific therapies others might not (Moras, 1998).

To answer the question of interest, the PsycINFO and MEDLINE databases were searched for English language, randomized outcome studies of behavioral therapies for alcohol, cocaine, or opiate abuse. The search was focused on behavioral therapies when used alone, although all the studies of opiate abuse found were of behavioral therapies combined with pharmacological interventions. In addition, the general substance abuse literature was searched for all gender-related findings in humans (e.g., epidemiological, course of disorder, biological responses to drug use). The outcome study search was focused on studies published between 1990 and May, 2000, but a few from the late1980s also were reviewed. The time limitation was used mainly because the mandate to include women in federally-funded studies occurred in the early 1990s. Before then, analyses of drug abuse treatment outcomes by gender were particularly rare, often due to relatively low percentages of women in samples and small sample sizes. The outcome studies of standard, mixed-gender behavioral therapies that were located were classified into three categories: gender difference outcome analyses reported and differences found; gender analyses reported and no differences found; gender analyses not mentioned.

CONCLUSIONS

Evidence on the need for gender-specific therapies comes from randomized outcome studies of: standard, mixed-gender behavioral therapies; women-specific interventions; and women-specific enhancements to standard therapies. Gender differences in recruitment, retention, relapse, and acute outcomes all are relevant to determining the need for gender-specific therapies. For example, gender differences in recruitment could indicate that a particular therapy is less acceptable to women than it is to men.

Standard, mixed-gender behavioral therapies

Nineteen randomized studies of standard behavioral therapies for cocaine or opiate abuse were located. None reported gender differences in outcomes. However, the results of outcome analyses by gender only were reported in about 20% of the studies. The majority (almost 80%) of cocaine and opiate abuse studies published in the last 10 years did not mention gender, other than the gender representation in the sample. Thus, it cannot be determined whether gender analyses were done but no differences were found or if the analyses were not done. The situation is somewhat different for alcohol abuse. Gender analyses were reported for a markedly larger proportion of randomized studies of behavioral therapies for alcohol abuse (about 85%) than for cocaine or opiate abuse. Gender differences, all favoring females, were reported in about 50% of those in which such analyses were discussed. Specifically, better follow-up results were found for women in three studies (Project MATCH, 1997; Sanchez-Craig *et al.*, 1989, 1991). (The gender difference finding in Project MATCH (1997) was not the one specifically hypothesized.)

The general paucity of reported gender differences in outcomes of standard mixed-gender therapies when used for cocaine, opiate, or alcohol abuse is strikingly discrepant with a large literature of both clinically-based hypotheses and research-based findings (e.g., on drug use patterns) that suggests gender is treatment-relevant. A variety of factors could be contributing to the discrepancy. For example, it is possible that gender *as a single attribute* is not a robust moderator of response to behavioral therapies or a robust marker for other, gender-related moderators. Almost no randomized outcome studies have been designed to test hypotheses derived from the gender difference findings of non-outcome research on substance abuse. The representation of women in study samples almost without exception is about 35% or less which reduces statistical power to detect gender differences. Also, as already noted, the results of tests for gender differences in drug use outcomes have been reported in only a small percentage of randomized studies of cocaine or opiate abuse.

Women-specific interventions

Women-specific treatments and women-focused enhancements to standard treatment programs for substance use disorders (e.g., day care services at treatment sites, vocational and parenting skills training, women only therapy groups, live-in option for a woman's children at residential treatment programs) are recommended and justified in a large, non-outcome literature that dates back to the 1970s (Davidson & Bemko, 1978; Hagan *et al.*, 1994; Hodgins *et al.*, 1997; McCaul & Svikis, 1999; Wald *et al.*, 1995; Wechsberg *et al.*, 1998). As of mid-2000, few randomized outcome studies have been done to test the recommendations. Four exceptions are Carroll *et al.* (1995), Dahlgren & Willander (1989), Hughes *et al.* (1995), and Volpicelli *et al.* (2000). All but Dahlgren & Willander (1989) were of female cocaine abusers who were either mothers or pregnant. Hughes *et al.* (1995) did not report substance abuse outcomes. Two of the remaining three found better substance abuse outcomes at follow-up for the woman-

specialized condition: one was a woman only, early intervention program for alcohol abuse (Dahlgren & Willander, 1989); one was an outpatient cocaine treatment program that offered several enhancements, some of which were women-focused (Volpicelli *et al.*, 2000).

Findings also suggest that some women-specific treatment enhancements can increase retention of women (Hughes *et al.*, 1995), and that women only programs can extend recruitment to subtypes of women who typically do not seek treatment in mixed-gender programs (Copeland *et al.*, 1993; Dahlgren & Willander, 1989).

Directions for treatment research

A basic recommendation is that investigators report the results of outcome analyses by gender in all studies of mixed gender behavioral therapies. Caveats that are needed to interpret results also should be included such as the power of the study to detect gender differences. Secondly, gender differences have been found in a variety of non-outcome studies of substance use disorders (e.g., Griffin *et al.*, 1989; Sklar *et al.*, 1999), a fact that is inconsistent with the weight of the evidence to date from outcome studies of standard, mixed-gender therapies. Thus, key questions for substance abuse treatment research include: Which gender difference findings might be treatment-relevant?, and What are their specific implications for developing or modifying behavioral therapies? A third suggestion concerns a data analytic strategy. Signal detection analysis (e.g., Kraemer, 1992), has been used to identify subgroups of treated nicotine patients who differ in their probability of responding (Smith *et al.*, 1999). Signal detection analysis might help reveal if and how gender is related to the outcomes of behavioral therapies for other types of substance abuse.

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REFERENCES: Available from author upon request (3535 Market Street, Room 641, Philadelphia 19104 or morask@landru.cpr.upenn.edu).

GENDER DIFFERENCES IN PHARMACOTHERAPY TRIALS FOR OPIATE AND COCAINE DEPENDENT INDIVIDUALS

H. E. Jones

The Johns Hopkins University School of Medicine, Baltimore, MD

Approximately three times as many men as women report using illicit drugs and four times as many men than women comprise the drug treatment program population. Although more men than women abuse drugs, substance abuse/dependence has been ranked as the second most common psychiatric disorder, especially in women of childbearing age (Robins *et al.*, 1984). Thus, it is critically important to better understand female substance users in order to identify effective treatments for them.

Women have different risk factors and reasons for initiation and continuation of drug use relative to men. Compared to men, women have lower levels of drug treatment entry, retention and completion (Nelson-Zupko *et al.*, 1995). One explanation for these differences may be found in the baseline characteristics. Compared to drug addicted men, women addicts are more likely to have more severe medical, psychological, social and employment problems and less severe legal problems (e.g., Kosten *et al.*, 1985). Drug use is the largest risk factor for contraction of HIV infection among women. Nearly 50% of AIDS cases in women result from intravenous drug use by the woman herself and an additional 20% result from intravenous drug use by her sexual partner(s).

Compared to men with substance abuse disorders, women with substance abuse disorders are more likely to have affective disorders, higher psychological distress levels (e.g., Kosten *et al.*, 1985), suicide attempt histories (Gomberg, 1989), a greater amount of psychopathology (Marsh and Simpson 1986), lower self-esteem, and fewer coping skills. Drug-abusing women have more relationship problems, are more socially isolated and have more difficulty socializing than male substance abusers (e.g., Wallen 1992). High rates of reported historical and recent sexual, emotional and physical abuse are present in the female drug-abusing population (e.g., Miller *et al.* 1989). One-third to one half of the substance-abusing women live with a substance abusing man (e.g., Kosten *et al.* 1985).

Moreover, drug-abusing women tend to be at an economic disadvantage to men in that they have less vocational training and job skills, and high rates of unemployment (e.g., Allen, 1995) and receive more public assistance (McCance-Katz *et al.*, 1999).

Although a number of important differences exist between men and women with substance use/abuse and/or dependence problems, it is not known if these differences occur in distinct sub-populations of opiate and cocaine dependent individuals. Thus, a study compared the pre-treatment characteristics of opiate dependent (N=220; 144 men and 76 women) and cocaine dependent (N=165; 91 men and 74 women) patients enrolling in an outpatient treatment/research clinic. Within cocaine and opiate dependent groups, men and women were compared on demographic characteristics, Addiction Severity Index (ASI) variables and DSM-IV Axis I and Axis II disorders. Groups were similar on race, legal, marital and employment status. Within the cocaine dependent group, women first used cocaine at a significantly older age, had a shorter lifetime use duration, began alcohol use later, had a shorter lifetime alcohol use duration, and used marijuana for a shorter time and less often than men. Women received more social service money, less illegal income and had more severe medical and psychiatric problems than men. Women had higher PTSD rates and borderline personality disorder whereas men had higher APD rates. In the opiate dependent group, women first used opiates later, sought treatment earlier and were less likely to have used alcohol and marijuana compared to men. Women had higher rates of lifetime and current major depression and PTSD and less APD than men. Thus, there are important differences between men and women within different substance dependent groups. Among women, cocaine dependent women especially may benefit from enhanced alcohol treatment and both cocaine and opiate dependent women may require psychiatric services (Jones *et al.*, 2000).

Since women appear to have unique treatment needs and may differ from men in their response to psychosocial treatments as well as medications to treat substance abuse, it is important to investigate gender differences. An examination of 17 reports of double-blind, randomized clinical trials examining opioid agonist pharmacotherapies including Levomethadyl acetate-hydrochloride (LAAM), methadone and/or buprenorphine for opiate dependence treatment published between 1976 and 1999 revealed three main observations. First, 13 of 17 (76%) clinical trials studies included women. Moreover, women comprised approximately 30% of the participant population in these trials. Women were included as participants in opiate agonist medication trials since 1988, which coincides with the 1994 NIH research regulations mandating that women be included in research. Second, although women have been included in clinical trials of opiate agonist medications to treat opiate dependence, only 5 of the 17 (29%) clinical trials reported specifically examining gender effects and only two of these trials included gender as part of the primary outcome analysis (Johnson *et al.* 1995; Eissenberg *et al.* 1997). The remaining three examined the effects of gender in secondary analyses (Schottenfeld *et al.* 1998; Jones *et al.* 1998; Strain *et al.* 1999). Interestingly, the third main observation is that when gender effects were examined, in three of the five cases significant differences were observed.

Although gender differences have been reported, their importance is not fully understood. However, the role of gender differences must be considered when employing these medications. Since most study results did not report gender differences, it is unclear as to whether this omission of gender effects was due to the fact that gender was included in the analyses and no significant differences were found or that gender differences were not examined. Future studies should, at a minimum, report whether or not gender was included as a factor in the analyses and whether or not there were gender differences. Further, future studies should undertake clinical trials using gender-sensitive models. A greater percentage of female participants should also be recruited into these studies by addressing many of the gender specific barriers that preclude women from participating (e.g., lack of child care, insensitivity to abuse issues, lack of gender specific treatment programming).

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SYMPOSIUM IV

ANTECEDENTS, CONSEQUENCES AND TREATMENT OF DRUG ABUSE: A COGNITIVE SCIENCE PERSPECTIVE

D. Shurtleff and E. London, Chairpersons

National Institute on Drug Abuse, Bethesda, MD, University of California at Los Angeles, Los Angeles, CA

The prefrontal cortex and mesostriatal system has an important role in many cognitive functions such as planning, working memory, decision-making and forms of inhibition. The role of these functions in determining both the vulnerability to and consequences of drug abuse needs to be explored in detail. Furthermore, cognitive science and drug abuse research have converged over recent years to provide valuable and complimentary perspectives for understanding drug addiction. This symposium provided the CPDD community the opportunity to learn about exciting new advances in cognitive science and drug abuse research. The speakers for this symposium presented (1) an overview of the role of the prefrontal cortex in higher order cognitive function, (2) the effects of drugs of abuse on cognitive abilities related to prefrontal cortical function, (3) discussed the relationship between deficits in higher order cognitive function and increased risk for substance abuse disorders (4) and discussed the role of higher order cognitive functions in treatment interventions. This symposium served to inform the audience of the current and potential role of cognitive science in understanding the antecedents and consequences of drug abuse and its role in the development of prevention and treatment interventions.

EXPLORING THE SOCIAL AND EMOTIONAL FUNCTIONS OF FRONTAL CORTEX

M. Farrah

University of Pennsylvania, Philadelphia, PA

The goal of this talk is to provide an overview of prefrontal function that reflects current thinking in cognitive neuroscience, and can serve as a context for the talks that follow. The talk began with the idea of a "frontal lobe syndrome," and the ways in which our understanding of prefrontal function has been refined through experimental research with patients and with neuroimaging of normal subjects. Although the abilities compromised by prefrontal damage have many commonalities, one to another, there does not seem to be a single element that distinguishes them, as a group, from functions not dependent on prefrontal cortex. Many, but not all, involve the ability to resist an easy, available or overlearned response. Many, but not all, involve the coordination of multiple elements of a complex task. Many, but not all, involve holding information about a stimulus or the task instructions in working memory for an interval of time. Finer grained analyses of PFC, aided by functional neuroimaging of normal subjects, has indicated some specialization within prefrontal cortex, with orbitomedial, dorsolateral, and anterior cingulated having at least partially distinct patterns of involvement with different tasks, but simple, principled relations between function and anatomy remain elusive. For example, while it is true that orbitomedial cortex is important for inhibition, the Wisconsin Card Sort test (which has been taken as a test of inhibition, in that the familiar just-used sorting criterion must be inhibited) is associated with dorsolateral cortex, and tasks that do not seem especially dependent on inhibition, such as weighing risks and benefits in a gambling situation are associated with orbitomedial cortex.

I then reviewed current theories of prefrontal function. Overall, these theories can be contrasted on the degree of unity versus modularity they hypothesize in prefrontal function. Variants of a central executive theory, including the Supervisory Attentional System of Shallice, attempt to explain a wide range of phenomena. Working memory theories may also attempt to account for many of the seemingly higher level impairments that follow frontal damage, by showing how working memory deficits can ramify through the system as it performs more complex tasks. Alternative approaches focus on one particular function, such as inhibition or, even more narrowly, the ability to use "somatic markers" of bodily state in decision making.

IMPAIRED COGNITIVE FUNCTION ASSOCIATED WITH CHRONIC DRUG ABUSE: A COMPARISON OF AMPHETAMINE AND OPIATE ABUSERS

R. Rogers

University of Oxford, Oxford, England

Chronic drug abuse may be associated with a wide range of neuropsychological deficits, some of which may reflect disrupted monoaminergic neuromodulation. In this talk, I reviewed some of our recent experimental work directly comparing the neurocognitive deficits of chronic amphetamine abusers and chronic opiate abusers, as well some complementary psychopharmacological studies that may indicate candidate neurochemical bases for some of these impairments. Specifically, our earlier studies found that chronic amphetamine abusers, but not chronic opiate abusers, were markedly impaired in the performance of a computerised decision-making task, previously shown to be selectively impaired by lesions of the orbital prefrontal cortex; and further, that this aspect of their altered cognitive profile can be modelled by reduced plasma tryptophan in normal control volunteers. More recently, we have found that chronic amphetamine abusers also show specific difficulties in the control of an attentional bias in a visual discrimination learning task believed to involve the functioning of the dorsolateral PFC. The implications of these results for our understanding of the underlying neurocognitive impairments in chronic drug abusers, and for further research and treatment development were discussed.

DOSE-RELATED COGNITIVE EFFECTS OF CHRONIC DRUG ABUSE

K. Bolla

Johns Hopkins Bayview Medical Center, Baltimore, MD

This presentation examined the dose-related association between chronic cocaine use and neurobehavioral performance. A battery of neuropsychological tests was administered to 30, 4 week - abstinent chronic cocaine abusers and 21 non-drug using controls matched for age, education, and intelligence. After controlling for age, education, and intellectual ability, greater use of cocaine (grams per week) was associated with larger decrements on tests measuring executive functioning, visuoperception, psychomotor speed, and manual dexterity. Dose-related effects were found primarily on complex tasks of higher cortical functioning involving an integration of multiple cognitive abilities (i.e., attention/planning/ mental flexibility - executive functioning, psychomotor functioning). The neurobehavioral substrate associated with these behaviors is probably the prefrontal cortex. Dose-related effects were found on Trails B, the Wisconsin Card Sorting Task (WCST), and a match-to-sample reaction time test. Due to their high sensitivity these tests generally detect decrements related to neurologic dysfunction. The Trails B and WCST tasks both involve cognitive flexibility, but the WCST also involves the ability to use feedback to monitor and change behavior. The computer administered Repetition of Numbers Reaction Time Task requires the participant to respond when a number presented on the computer monitor is identical to the number preceding it (match-to-sample). The heaviest cocaine users showed slower median reaction times and made more errors of omission (true positive) and commission (false positive). False positive errors may reflect a tendency to be impulsive. Dose-related decrements were also found in attention/concentration. For example, heavier cocaine use was related to lower performance on the cancellation test for randomly placed symbols, a measure of attention and concentration. In addition, adverse associations were found between cocaine use and performance on a memory test that is sensitive to attentional problems (RAVLT Trial 1). These results suggest that chronic cocaine use is associated with persistent decrements in cognitive function that are most pronounced in heavy users. Knowledge of specific cognitive processing deficits in chronic cocaine abuse would be useful for designing individually tailored drug treatment programs.

EXECUTIVE COGNITIVE FUNCTION, ANTISOCIAL BEHAVIOR AND DRUG ABUSE

P. Giancola

University of Kentucky, Lexington, KY

Low executive functioning has been found to be related to increased drug and alcohol use and an increased likelihood of developing a substance use disorder (SUD; including alcohol) (Giancola and Tarter 1999). Executive functioning can be defined as the planning, initiation, and self-regulation of goal-directed behavior. Our research has shown that pre-adolescent boys with a family history of an SUD show lower executive functioning compared with those with a negative family history of disorder (Giancola *et al.*, 1996). It has also shown that female adolescents with an SUD show lower executive functioning compared with controls (Giancola *et al.*, 1998). These data suggest that low executive functioning may be a risk factor for pathological drug abuse.

Other research we have conducted has indicated that low executive functioning is related to increased disruptive, delinquent, and violent forms of anti-social behavior in persons at high risk for an SUD (Giancola *et al.*, 1996), those with an SUD (Giancola *et al.*, 1998a), those with a conduct disorder (Giancola *et al.*, 1998b), and in normal controls (Giancola and Zeichner,1994). Other than past drug use, it has also been found that antisocial behavior is one of the strongest predictors of drug use and an SUD diagnosis (Biederman *et al.*, 1997; Wood *et al.*, 1995). As such, it has been hypothesized that heightened antisocial behavior may serve as a functional mechanism between low executive functioning and increased drug use (Giancola and Tarter, 1999).

A recent prospective study conducted at our research center using a sample of boys with and without a family history of an SUD found that low executive functioning at age 10 – 12 predicted antisocial behavior at age 14 which then predicted drug use at age 16 (Giancola and Parker 2000). There was no direct relation between executive functioning and drug use suggesting that antisocial behavior may be a necessary functional mechanism predisposing toward drug use.

SYMPOSIUM V

HIV PREVENTION INTERVENTIONS TARGETING DRUG USERS - CURRENT STATE OF THE ART AND STATE OF THE PRACTICE

R. E. Booth and J. W. Curran, Chairpersons

OVERVIEW AND RECOMMENDATIONS

J. W. Curran

Rollins School of Public Health, Emory University, Atlanta, GA

It is now the twentieth year since the discovery of the occurrence of AIDS and over sixteen years since HIV was proven to cause the disease and diagnostic testing became available. The two decades have seen much progress in understanding the disease and in antiviral therapy so that, for the majority of infected persons in the wealthiest countries, great improvements in survival and well-being are being seen. The epidemic has progressed tragically in developing countries in Africa, and parts of Asia, Latin America, and the newly emerging States of eastern Europe, however, and the scientific advances have had little impact there.

In the United States, approximately 40 percent of HIV infections are directly or indirectly attributable to the use of injecting drugs. This includes the majority of cases in women, children, and African-American and Hispanic minorities. Progress in prevention research for these populations has occurred as described in the studies below but implementation of effective measures has been far from complete.

Several patterns are noted from the studies: Kwiatkowski and Booth note that the use of indigenous(peer) outreach workers has been consistently effective in reducing HIV risk in drug using populations. Both Sterk and colleagues and Wechsberg and colleagues indicate the importance of listening to specific populations at risk and designing and implementing interventions with their specific needs in mind. They note, in particular, the specific issues confronting minority women and those of low educational status. Metzger summarizes the considerable body of information which demonstrates that substance abuse treatment itself is an effective HIV prevention measure. In fact, it stands to reason that recognizing and dealing with the individual's addiction would be necessary in order to reduce their risk of acquiring or transmitting HIV. This again reinforces the point that HIV prevention interventions must be tailored to the needs of those at risk. Des Jarlais presents compelling evidence supporting the effectiveness of needle exchange programs in reducing the spread of HIV, despite the unwillingness of the federal government to provide financial support. The "second generation questions" he raises include: Why are some syringe exchanges more effective than others? and How should syringe exchange programs be integrated into comprehensive programs of HIV prevention? This latter question includes integration of syringe exchange programs with drug abuse treatment programs. We are still far from answering these second generation questions, but the working hypotheses can be summarized as "services should be user-friendly" and "the more services the better."

Finally, the struggle against the worldwide HIV epidemic faces several common barriers: denial, discrimination, and scarcity...and these are manifest clearly in the HIV epidemic among substance abusers. Individuals and even providers can deny the high risk of HIV infection, including the risk from unprotected sexual behavior. Discrimination against those with HIV infection persists in our society alongside our prejudices against minorities and the poor. The societal discrimination against those who abuse substances is clearly reflected in policies favoring incarceration over treatment and the lack of parity in public and private insurance for substance abuse treatment. Only with this "societal discrimination" background can we understand the continued strong political resistance to expansion of drug treatment and implementation of needle and syringe exchange as part of a more comprehensive program. For the world and the underserved everywhere, the largest barrier remains scarcity of resources. In developing countries, scientific progress against HIV provides little hope thus far due to the lack of health resources available. For prevention of HIV in drug users worldwide, effective approaches often are seen as competing with each other or with other needs in poor communities. Commitment to prevention research and to advocacy for the HIV prevention measures proven effective is necessary to make further progress against the epidemic.

STATE OF THE SCIENCE AND STATE OF THE PRACTICE FOR NEEDLE EXCHANGE PROGRAMS

D. C. Des Jarlais

Chemical Dependency Institute, Beth Israel Medical Center, New York, NY

Syringe exchange continues to be controversial in the United States. Despite official findings by the Surgeon General that syringe exchange programs do reduce HIV transmission and do not lead to increased drug use, there is still no federal funding of syringe exchange programs in the country. The number of US programs has continued to increase, however, and there are now 157 programs operating in the country. Approximately half of these programs have some form of local or state government support.

Research on syringe exchange has moved from the "first generation questions" of whether the programs lead to increased illicit drug use and whether they lead to reductions in HIV transmission. The answer to the first question is a clear no. There is no evidence to date of any increase in illicit drug use associated with syringe exchange. The answer to the second question is "usually, but not always." In the great majority of studies (13/16) that used HIV infection as an outcome measure, syringe exchange was associated with low rates of HIV transmission among injecting drug users. Research now addresses a "second generation" of questions aimed at determining how to make needle exchange programs maximally effective in a variety of settings with differing environmental and social constraints.

HIV RISK REDUCTION INTERVENTIONS: GENDER, RACE, SKILLS AND MOTIVATION

C. E. Sterk, K. W. Elifson, and D. Kidder

Rollins School of Public Health, Emory University, Atlanta, GA

The objectives of this presentation were: (1) to address the need for gender and race-specific interventions, with an emphasis on interventions targeting female drug users; (2) to present data on formative research to design an HIV risk reduction intervention for African-American female crack cocaine users and on the preliminary results of an ongoing HIV risk reduction tailored to these women. The number of new HIV/AIDS cases is growing fastest among African-American women in the Southeastern United States, although few prevention and intervention programs are in place that target their specific circumstances and needs. The need for women-specific interventions has shown to be related to a number of factors in the lives of female drug users. The most salient factors are the dynamics surrounding the steady relationships of female drug users, the link between sex and crack cocaine use, and gender differences in drug use experiences with women often being introduced to drugs by men, being more stigmatized than male users, and becoming dependent more quickly than men. Race-specific interventions focused on African Americans are needed to address prominent themes such as the role of the extended family, the importance of religiosity and spirituality as coping strategies, the history of the role of African-American women in the United States, and racism in our society.

Data collection was initiated in June 1997 and is ongoing in Atlanta, GA. The first year consisted of formative research among 90 women. Data were collected through open-ended, in-depth interviews, observations and mapping. Based on the formative study, a woman-focused HIV risk reduction intervention for African-American crack users was designed and implemented. The formative interventions revealed the importance of placing the HIV epidemic in the local context, to take a holistic perspective and not to focus on the women as drug users only, to place HIV in the context of other past and current events in the women's lives, and to link the risk reduction intervention to other social and health services. In addition, the formative research showed the importance of providing the intervention in a safe place that has flexible hours, to provide individual as well as group activities, and to allow the clients to gain ownership of the program.

The interventions to be evaluated consisted of the NIDA standard intervention for HIV risk reduction among drug users, an enhanced intervention condition focused on motivation and built around motivational interviewing, and an enhanced intervention condition focused on negotiation and conflict resolution skills. The NIDA standard consisted

of two client-centered HIV counseling and testing sessions, with specific mention of race, gender, and crack cocaine use. The Motivation Intervention included the NIDA standard and two additional sessions focused on assisting the women to identify which changes they wanted to make in their life and how to set realistic expectations. The Negotiation Intervention also included the NIDA standard and two additional sessions which emphasized technical skills (e.g., condom use), communication skills, and conflict resolution skills. Subsequent data collection consisted of process information as well as baseline, post intervention, and 6-month follow-up interviews to assess behavioral change supporting HIV risk reduction.

The sample to date consists of 315 women, all of whom were active crack cocaine users, African-American and lived in Atlanta. All women were out of drug treatment or any other institutional setting at the time of their enrollment into the project. Their mean age was 37.2 years and their mean level of educational attainment 11.4 years. The majority (92.1%) were mothers. The mean age of first vaginal intercourse was 15.3 years. In terms of the number of partners by type in the past year, the mean for steady partners was 1.1 and for paying partners 3.6. Overall the mean number of sex partners in the past year was 20.4. Over three-fourths (86.8%) of the women had encountered physical or sexual abuse at some in their life.

Due to the eligibility criteria for enrollment into the study, all women smoked crack cocaine at the time of their enrollment. At the post intervention assessment, the percentage of women who did not smoke crack cocaine in the last 30 days increased for all three intervention conditions, with the largest increase among women in the standard condition. When exploring behavioral change in terms of bartering sex for crack, women in all three intervention conditions improved as well. However, we also learned that women in the standard intervention –the group with the largest increase in number of days of being crack free in the last 30 days– reported more sex for crack bartering on those days that they used crack both at the post intervention and the 6-month assessment. Smoking crack cocaine in the context of sex was also reduced for women in all three conditions, however, long-term behavioral change was less likely to be sustained among women in the standard condition. Regarding changes in sexual behaviors, women in all three intervention conditions reported increased levels of consistent condom use for vaginal sex with steady as well as paying partners, with no long-term differences between the three groups. In terms of psychological characteristics, women in the motivation intervention reported greater positive change, with an increase in self esteem and a decrease in depression and anxiety.

We also detected some unintended changes. Although the focus of the intervention was to reduce HIV risk and not on ceasing drug use or seeking drug treatment, a significant number of women did enter drug treatment or joined self-help groups such as NA. Our process data also reveal that many women, when asked to describe the impact of their involvement in the program, emphasized feeling less marginalized and more integrated into mainstream society. This often was due to having been able to get a photo ID, qualifying for Medicaid, or developing trust in program staff such as the interventionists. When evaluating tailored risk reduction intervention programs, it is important to focus on outcome measures, process data, as well as unintended outcomes.

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PREVENTING HIV INFECTION: THE POTENTIAL OF SUBSTANCE ABUSE TREATMENT

D. S. Metzger

VA Center for Studies of Addiction, University of Pennsylvania, Philadelphia, PA

We are now moving through the third decade of the AIDS epidemic among IDUs in the United States. Over 120,000 people have died as a direct result of transmission via contaminated injection equipment and its estimated that over 300,000 are infected or living with AIDS. Since recognition of the AIDS epidemic among IDUs in the early 1980's, a range of interventions have been implemented—with various levels of enthusiasm and support-- in an effort to prevent HIV infection among drug users and those with whom they come in contact. These include 1) educational programs; 2) outreach to drug users not in treatment 3) increased access to sterile injection equipment, and 4) drug treatment. No other strategy has been as widely endorsed nor as frequently evaluated as substance abuse treatment. The data suggest that the underlying mechanism of protective action is rather simple--individuals who enter effective treatments reduce their drug use. This lower rate of use leads to fewer instances of drug related risk behavior. This lower rate of exposure results in fewer infections with HIV.

Most of the research work examining risk reduction has focused on methadone treatment, the modality that serves opiate dependent individuals. This is not surprising as heroin is the drug most often associated with injection related HIV transmission. Nowhere is the association between treatment involvement and reduction in drug use more visible than in the results of study by Ball and his colleagues in which IDUs in methadone treatment retrospectively reported on their injection behaviors. As shown, universal rates of injection begin to show dramatic decline following entry into methadone treatment. This decline continued through the four years of study. Importantly, the reduction is not immediate, nor is it universal. After four years, twenty percent of the subjects report continued injection.

More recently, Booth *et al.* reported on the results of comparisons of over 200+ subjects who subsequent to their baseline assessment entered and remained in treatment for at least 90 days. These subjects were members of a cohort of nearly of 3000 IDUs who were all recruited while out-of-treatment. Thus the data is prospective and as such a bit more powerful. When compared to those who remained untreated, those who had entered treatment reported injecting heroin, cocaine, and speedball significantly less frequently than those subjects who remained out of treatment. Significant reductions in crack use were also noted. Additionally those who had been in treatment for 90 days or more were three times more likely to report no drug use and nearly four times more likely to provide a urine specimen with no detectable drugs.

Do these reductions in drug use lead to reductions in HIV risk behavior? Two recent reviews of the literature on this question conclude that they do. In our 1998 review, 17 studies Published between 1984 and 1997 were reviewed and we found the data to be quite consistent in reporting that risk behaviors are lower among those in treatment relative to their pre treatment risk behaviors, their post treatment risk behaviors, and untreated counterparts. In fact when Capplehorn and Ross calculated odds ratios for needle sharing among methadone treated individuals from nine studies, dramatically lower rates were reported. Jim Sorensen has an article in the current issue of Drug and Alcohol Dependence in which 33 articles published between 1985 and 1998 were reviewed. They conclude that subjects in treatment reduce risk over time and that treated subjects have lower risk than untreated subjects. Both reviews also concluded that treated subjects have lower prevalence and incidence of HIV infection. These data are very convincing in confirming the association between treatment participation and lower rates of drug use and risk behaviors.

In Philadelphia, a prospective longitudinal study of HIV infection and risk behaviors among in and out-of-treatment drug users was initiated in 1989. In this study, 152 IDUs were randomly selected from a methadone treatment program and 103 out-of-treatment IDUs were recruited using a chain referral technique. Those who remained out-of-treatment were nearly seven times more likely to have become infected than were those who remained in treatment during the first eighteen months of the study. Among those who remained in methadone treatment for the entire 18 month study period, 3.5% became infected. Among those who remained out-of-treatment, 22% became infected with HIV (Metzger, *et al.*, 1993).

Numerous studies have documented that significantly lower rates of risk behaviors are practiced by drug users who are in treatment. This has been the finding when in-treatment IDUs were compared to untreated IDUs, when drug use patterns during treatment were compared to pre-treatment patterns, and when drug use patterns during treatment were compared to post-treatment drug use practices. Importantly, these self-report behavioral findings are consistent with most studies that have examined HIV infection rates. The consistency of these findings suggests that increasing access to treatment is a legitimate and necessary HIV prevention activity. It is also clear that treatment is important, but by itself, insufficient to protect the health of the drug using community. Not all users are interested in treatment, able to gain access to treatment, or able to remain in treatment and not all of those who do enter treatment eliminate their use of drugs. HIV prevention will therefore necessarily require the integration of treatment with outreach and harm reduction strategies in order to help protect the health of drug users and the larger community.

In sum, these are some of the data that have for the past fifteen years helped to illuminate the association between drug treatment participation and HIV prevention. The data are consistent and they are impressive.

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OUTREACH AS EFFECTIVE HIV RISK REDUCTION AMONG DRUG USERS

C. F. Kwiatkowski and R. E. Booth

Department of Psychiatry, University of Colorado Health Sciences Center, Denver, CO

This presentation summarizes the results of the past thirteen years of research conducted through Project Safe, a community-based outreach intervention project at the University of Colorado in Denver. Project Safe was founded in 1987 by Robert Booth, Ph.D., as one of three sites participating in a NIDA-funded Demonstration Project to study the effectiveness of street-based outreach interventions in reducing HIV risk behaviors among drug users. This was followed in 1990 by Project Safe's involvement in NIDA's Cooperative Agreement for AIDS Community-Based Outreach Intervention. In 1995, a grant was awarded to study the impact of treatment facilitation efforts on drug treatment entry, and the effectiveness of treatment in reducing risk behaviors.

The Indigenous Leader Outreach Model (ILOM) has been the cornerstone of Project Safe's intervention program. The goal of the ILOM is to reduce the spread of HIV among injection drug users and their sex partners. Outreach workers, indigenous to the community, are employed to serve as health educators and role models. Their mission is to increase HIV awareness and education in the community, help individuals assess their personal level of risk, provide alternatives to high risk behaviors and reinforce behavior change. In addition to HIV education, outreach workers distribute prevention materials such as bleach kits and condoms and provide social service referrals, including drug treatment. Individuals who agree to participate in research studies are interviewed at baseline by independent interviewers, receive ILOM-based educational interventions and HIV testing and counseling and are re-interviewed six months later.

Nearly 3,000 subjects have participated in Project Safe research studies over the past thirteen years. Although their characteristics vary across studies, they can generally be summarized as follows. Approximately two-thirds are male, averaging 35-40 years of age. In general, ethnicity is fairly evenly distributed across African American, Latino and white. Nearly half of the subjects have not graduated from high school and approximately one-third report being homeless. Nearly two-thirds are unemployed or disabled, almost all have been arrested (90%, on average) and they have been injecting drugs for an average of 15-20 years.

The Demonstration Project, which was conducted in three cities, revealed a number of important findings. First, that drug users can be located through street outreach, that they will discuss their risk behaviors in a structured interview setting and that they can be re-located for follow-up interviews. Second, when the ILOM intervention is implemented as designed, it is an effective means of reducing HIV risk behaviors. The second project, the Cooperative Agreement, employed a more rigorous experimental design by comparing HIV testing and counseling (NIDA's standard intervention) to a variety of site-specific enhanced interventions delivered to street-recruited drug users. This research confirmed the effectiveness of NIDA's standard intervention in reducing risk behaviors but did not support an added effect of more time- and resource-intensive enhanced intervention. In the third and most recent

project, several services were added to the standard intervention to facilitate subjects' entry into drug treatment. These included rapid, free intake appointments (no waiting period) and transportation to the intake appointment for all study participants who desired drug treatment. Participants were also randomized to receive a coupon for 90 days of free drug treatment. Results indicated that overall, treatment entry was greatly enhanced by the added incentives, and the free treatment coupon was extremely effective in improving treatment entry and retention.

In summary, the ILOM intervention and office-based HIV testing and counseling are effective means of reducing HIV risk behaviors among injection drug users. More expensive, time-intensive interventions have not been shown to be more effective than this basic approach. Outreach intervention combined with treatment facilitation is an effective method of moving drug users into treatment, particularly when coupons for free treatment are provided. This research has implications for widespread community-based outreach intervention programs and calls for enhanced collaboration between outreach and drug treatment programs to reduce the spread of HIV among drug users and their sex partners.

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WOMEN-FOCUSED HIV PREVENTION FOR AFRICAN AMERICAN CRACK USERS

W. M. Wechsberg, W. Zule, R. Perritt, A. Roberts, R. Middlesteadt, and A. Burroughs

Research Triangle Institute, Research Triangle Park, NC

HIV infection has been progressing rapidly among African-American women in the Southeast, particularly among women substance abusers. Although there is convincing evidence that women's risk differs from men's, particularly with trading sex for drugs among crack abusers, there are as yet no data indicating that an Afrocentric intervention targeting out-of-treatment women will be effective in changing the drug and sexual behaviors that put them at risk for HIV infection. The National Institute on Drug Abuse (NIDA) is sponsoring this 4.5-year study to determine whether a woman-focused HIV intervention can be more effective than a generic intervention at engaging women, reducing their drug use and risky sexual behaviors, connecting them with community resources, and getting them into treatment. The project has a three-group design consisting of a women-focused group (receiving a contextually rich and culturally sensitive intervention), a standard group (receiving the NIDA standard HIV intervention), and a control group.

The NC Women's CoOp specifically conducts outreach to African-American crack-abusing women that includes a rigorous intervention design, a cost-effectiveness study, and a process study. The woman-focused intervention was developed with women of the community and addresses drug dependency as "bondage," and supports recovery. It assists women in developing personal goals of independence and personal power in making choices, specifically around sexuality; role playing new skills and then acting on them; and developing positive social supports to maintain personal objectives. Although, the Women's CoOp builds upon many of the models and theories of HIV prevention, e.g., social cognitive theory, stages of change, health belief model, and social learning theory, it is grounded in feminist theory. It validates our objectives for women to 1) acknowledge how diverse they are, 2) help them recognize the changing nature of their lives, 3) enhance their awareness of the contextual pressures they are confronted with everyday, and finally, 4) help make changes by emphasizing the need to become more empowered by developing skills to be in control of their lives.

The Women's CoOp study takes place in two inner city locations in Durham and Raleigh, North Carolina: one in the basement of an African-American church located in an area where drug-related violence has occurred, and the other within walking distance of city shelters and soup kitchens. In fielding this large community-based study, we had to address a number of barriers for women to stay involved (e.g., transportation, homelessness, child care, getting them to treatment). However, we have reached 429 African-American women, with a mean age of 36. Half of these women are single, 47% report less than a high school education, two-thirds are unemployed, and 7% tested positive for HIV antibodies. Only 28% live in their own residence, with 34% reporting being homeless. While 74% report they have children, only 30% have children currently living with them. Nineteen percent had first sex at age 12 or earlier, 40% between 13 and 16 (17% did not comment), and 35% at age 16 through 18. Seventy-two percent reported a history of physical abuse (34% before age 16) and 50% reported a history of sexual abuse (55% before

age 16). Fifty-nine percent reported having traded sex for drugs. There was substantial variation in both the average length of a crack "run" and in the substances used to ameliorate the "crack crash," with certain substances such as alcohol, posing serious health hazards. Thirty-three percent smoked crack from 1 to 6 hours, 20% from 7 to 24 hours, 25% 1 to 5 days, and 7% smoked crack longer than 5 days. Thirty-eight percent of the women reported using alcohol to come down from their crack crash, only 12% reported smoking marijuana, 28% used nothing. Many women (35%) reported drinking water or milk, eating, or sleeping as their method of coming down from crack.

These women reported numerous kinds of sexual risk. Although 49% reported one sex partner, 12% reported one or more injecting-drug-user partners, and 4% reported more than 10 partners. Forty-one percent used alcohol one or more times during sex, 64% reported crack use one or more times, and 22% used marijuana during sex. There was a total of 1,225 traded sex acts for drugs reported in the past 30 days, with 49% of them protected. There was a total of 1,586 sex acts traded for money, food, or shelter in the past 30 days, with 55% protected. Over 20% of all the women reported a current sexually transmitted disease (STD) symptom. In general, 44% of the women believed that they were at-risk for HIV through both drug use and sexual behavior.

Preliminary Outcome by Groups. The first follow-up was three months after randomization: preliminary results for 221 women will be discussed here. A decrease in days of alcohol use was reported in all groups (control, standard, and women's) and a decrease in days of crack use in all groups was reported, with the largest reduction in the women's groups. The control group decreased days of crack use by 5 days (25%), the standard group by 3 days (19%) and the women's group by 7 days (38%). The control group reduced the number of unprotected sex acts by 3.5 acts (46.5%), the standard group by 1 act (15.5%), and the women's group by 2 acts (31.5%). Between baseline and 3-month follow-up, the mean score on the peer-support or not-using-drugs scale decreased by 11% (from 6.1 to 5.4) in the control group and by 3% (from 6.2 to 6.0) in the standard group, but in the woman-focus group it increased by 19% (from 5.7 to 6.8). The percentage of women reporting STD symptoms decreased from 18% to 11% in the control group, from 24% to 14% in the standard group, and from 23% to 13% in the woman-focused group.

At 3-month follow-up, criminal behavior was much more common among the control group than among either of the intervention groups. Nineteen percent of the control group members reported dealing drugs in the preceding 30 days, compared to 10% of the standard group, and 8% of the woman-focused group. There were similar differences in receiving stolen property, with 15% of the control group, 7% of the standard group, and 4% of the woman-focused group reporting receiving stolen property in the previous 30 days. The pattern for shoplifting was slightly different, with 10% of the control group, 1% of the standard group, and 4% of the women's group reporting shoplifting in the previous 30 days at follow-up.

Implications. This NIDA funded project is reaching African-American crack-abusing women who are homeless and trading sex. Furthermore, these women have high levels of co-morbidity and abuse histories that will be considered in the final analysis. However, it must be acknowledged that significant reductions in drug use and unprotected sex are occurring within all groups and, curiously, in the control group. When these women were interviewed after the follow up, they commented on how "excited" they were to be a part of this...they made comments as "love the staff...the study is important. . ." Indicating that, not only can we question whether instrumentation might have an effect, but the engaging staff who conduct the data collection — and the comfortable site with things for children to do, meals, and clothes to warm their daily life — may also have an effect.

This study is just beginning to understand what the real impact and cost will be. Longer follow-up is under way, and the sample is expanding. The costs and cost effectiveness analyses will be conducted after all field operations are complete. Hence, there is a need to consider an intervention that will not only meet the needs of African-American women substance abusers in the inner city, but a need to reach these women earlier in their drug using and sexual trading careers. Furthermore, brief interventions where women can be found in the community can be effective, even minimally; however, long-term sustainability is still in question.

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SYMPOSIUM VI

THE EFFECTS OF ACUTE AND CHRONIC OPIATES ON RECEPTORS AND SIGNAL TRANSDUCTION SYSTEMS

W. L. Dewey and S. Welch, Chairpersons

Virginia Commonwealth University, Richmond, VA

OPIATE ANALGESIA: CORRELATING THE MOLECULAR BIOLOGY IN BEHAVIOR OF PAIN CONTROL

G. W. Pasternak

Laboratory of Molecular Neuropharmacology, Memorial Sloan-Kettering Cancer Center, New York, NY

Classical pharmacological approaches have predicted three major classes of opioid receptor: mu, delta and kappa[1]. The years have held up this classification of opiate receptors. Opiate receptors were first demonstrated in binding assays almost thirty years ago and binding assays are now available for all three classes of opioid receptors[2]. Mu drugs have been examined most extensively since they represent the vast majority of clinically useful agents. They all share many characteristics. First, they show high affinity and selectivity in the mu opioid receptor binding assays. Second, they have similar pharmacological profiles, producing analgesia and a number of other actions, including respiratory depression and constipation. All these features implied a common mechanism of action. Yet, this is not consistent with their pharmacology.

Like morphine, most clinical opioids have been classified as mu analgesics. However, morphine differs from many of them pharmacologically, both clinically[3] and in animal models[4]. Patients in whom morphine is not effective or who are unable to tolerate it due to severe side-effects such as nausea/vomiting often find pain relief with minimal side-effects from a different mu drug. The observation of clinical incomplete cross-tolerance among mu opiates has provided yet another example of differences among these agents. When patients highly tolerant to one opioid are switched to another, analgesic activity oftentimes can be regained with the second drug at doses 50-75% lower than those predicted from equivalency studies in naïve patients. Indeed, if the dose of the second drug is not reduced, the patient may end up being overdosed. These observations are not consistent with a single, common mechanism of action, raising the possibility of multiple subtypes of mu receptors.

Initial studies suggesting subtypes of mu receptors date back several decades and were based upon binding experiments studies with novel antagonists that could selectively block certain actions of morphine and not others[5,6]. The unique activities of morphine-6β-glucuronide (M6G) have led to yet another mu receptor subtype[4]. In animal models, M6G showed incomplete cross tolerance to morphine[7]. Other approaches also supported differences in its actions. CXBK mice are insensitive to morphine given either systemically or supraspinally. Yet, these same mice respond normally to a number of other mu analgesics, including M6G, heroin and fentanyl. Thus, these models support the presence of three class of mu receptor subtypes.

The first mu opiate receptor, MOR-1, was first cloned in 1993[8,9]. It possessed many of the characteristics anticipated for these receptors based upon the selectivity of binding for various opiates. Furthermore, the receptor was identified within the nervous tissue and found to have distributions similar to those previously established for mu opiate receptors in more traditional receptors assays, such as autoradiography. Yet, the identification of a single gene encoding a mu opiate receptor left many questions regarding its relationship to mu opiate receptors mediating analgesia and the concept of multiple mu opiate receptor subtypes. Antisense studies based upon the sequence of MOR-1 clearly showed its importance in morphine analgesia[10,11]. Yet, antisense mapping studies looking at different exons within the protein revealed differing actions for morphine as opposed to M6G[11]. Probes based upon exon 1 of MOR-1 blocked morphine, but not M6G while other probes targeting exon 2 blocked M6G analgesia but not morphine. Thus, even studies at the molecular level, there was a suggestion that morphine and M6G acted through distinct mechanisms. This was further supported by observations in a knockout animal with a disruption of exon 1[12]. As anticipated, these animals were unresponsive to morphine at doses greater than 10-fold higher than its ED_{50} in wildtype controls. Yet, the mice still responded to M6G and other mu opiates, including heroin and

fentanyl. The continued sensitivity of M6G analgesia to an antisense against exon 2 of MOR-1 demonstrated that its response was still mediated by a MOR-1 gene product.

What was the relationship of the MOR-1 gene with the proposed mu receptor subtypes? Soon after the initial cloning of the mu opiate receptor, splice variants were identified and the gene expanded from four to five exons [13,14]. More recent work from our group has identified at least ten exons within MOR-1 which combine to generate at least seven different MOR-1 splice variants [15]. Each shows the same selectivity and high affinity for mu opioids as MOR-1. Additional evidence suggests even more variants may be associated with the MOR-1 gene.

The relationship of all these variants to the receptor subtypes predicted from the pharmacological studies is still unclear. However, their existence strongly supports the possibility that MOR-1 variants may be responsible for the complex pharmacology of mu analgesics described earlier.

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REGULATION OF UPLOAD RECEPTOR SIGNALING

H. Loh

University of Minnesota, Minneapolis, MN

The existence of multiple opioid receptors and the isolation of the endogenous opioid peptides in the brain, make it clear that the activities of these receptors can be regulated at various levels. The distinct brain regional distribution of the receptor suggests a tight transcriptional regulation. Early findings of alterations in receptor binding associated with tolerance to opioids implies that the receptor life cycle can be influenced by the presence of agonists. Until the recently reported cloning of opioid receptors, the detailed studies of the molecular mechanisms involved in their regulation could not be conducted. With the availability of the cDNA clones of the μ, δ- and κ-opioid receptors, and the elucidation of their gene structures, investigated opioid receptor regulation at various levels, and identified the specific receptors involved in the pharmacological actions of the opioids. It is now also possible to define the receptor domains responsible for the opioid ligand selectivities, agonist activation, and agonist-induced inactivation. Summarized in this presentation are our past efforts in defining the regulation of opioid receptor activities. Heterologous expression techniques, mutational analysis of receptors to characterize transcriptional elements, and the in vivo manipulation of the receptor gene levels have made it possible to determine the mechanisms whereby these receptors are regulated.

More specifically, we have previously reported the presence of dual (distal and proximal) promoters in mouse μ-opioid receptor (mor) gene, with mor transcription in mouse brain predominantly initiated by the proximal promoter. Sp factors, bound to double-stranded (ds) *cis*-regulatory elements, are critical for proximal promoter activity. WE now present evidence that a single-stranded (ss) *cis*-regulatory element and trans-acting protein factor are also important for proximal promoter activity. A 26 bp mor polypyrimidine/polypurine region (Ppy/u) can adopt as DNA conformation, as demonstrated by S1 nuclease sensitivity. Using electrophoretic mobility shift analysis with nuclear extracts from *m*or-expressing SH-Sy5Y cells, we demonstrate that the sense strand of PPy/u interacts with a major nuclear protein, termed mor polypyrmidine binding protein (mPy), which is not related to Sp factors. Southwestern blot analysis indicated that mPy protein is approximately 25 kDa in size. Functional analysis suggests that mPy protein can *trans*-activate mor promoter as well as a heterologous promoter. Moreover, combinatorial activation of ss (mPy) and ds (Sps) DNA binding factors, interacting with an overlapping DNA (Ppy/u) region, is necessary for proximal promoter activation. Thus these results results suggest that transcription of mouse mor gene is regulated by an interplay of ss and ds DNA binding factors. Discussion of these and related findings were presented, as well as hypotheses directed toward how these findings help us understand opiate actions.

CHRONIC DRUG EFFECTS ON OPIOID RECEPTOR/G-PROTEIN INTERACTIONS IN BRAIN

S. R. Childers, L. J. Sim-Selley, C. E. Maher, and T. J. Martin

Department of Physiology/Pharmacology and Center for the Neurobiological Investigation of Drug Abuse, Wake Forest University School of Medicine, Winston-Salem, N.C. and Department of Pharmacology, Virginia Commonwealth University, Richmond, VA

Chronic heroin use produces high levels of tolerance and physical dependence, but the biological mechanisms which mediate these effects in brain are not clear. Heroin is a member of the family of opiate alkaloids which exert their actions as antinociceptive and addictive agents by binding to mu opioid receptors. These receptors belong to the superfamily of seven transmembrane spanning receptors that couple to $G_{i/o}$-proteins (Chen *et al.* 1993). The ability of receptors to activate Gα can be measured by agonist-stimulated [^{35}S]GTPγS binding (Traynor and Nahorski 1995). The more recent development of [^{35}S]GTPγS autoradiography (Sim *et al.* 1995) has provided a method to determine the neuroanatomical localization of receptor-activated G-proteins. Previous studies from our laboratory using [^{35}S]GTPγS autoradiography showed that chronic morphine treatment for 10-12 days produced decreases in mu opioid agonist G-protein activation in specific brainstem nuclei (Sim *et al.,* 1996), with no change detectable in forebrain structures. The present studies expand on these observations by showing: 1) desensitization in midbrain and brainstem structures following chronic heroin self-administration, accompanied by an actual increase in mu receptor binding sites; 2) loss of mu receptor/G-protein coupling in brain membranes, showing a loss of agonist efficacy rather than potency; 3) the time course of mu receptor desensitization is slow compared to that of analgesic tolerance development, with desensitization occurring first in brainstem nuclei.

First, mu opioid receptor binding and receptor-activated G-proteins were examined following chronic heroin self-administration. Rats were trained to self-administer i.v. heroin for up to 39 days, achieving heroin intake up to 366 mg/kg/day (Sim-Selley *et al.,* 2000). Mu opioid-stimulated [^{35}S]GTPγS and [^{3}H]naloxone autoradiography were performed in adjacent brain sections. Agonist-stimulated [^{35}S]GTPγS autoradiography also examined other G-protein coupled receptors, including delta opioid, ORL-1, $GABA_B$, adenosine A_1, cannabinoid and 5-HT_{1A}. In brains from heroin self-administering rats, decreased mu opioid-stimulated [^{35}S]GTPγS binding was observed in periaqueductal gray, locus coeruleus, lateral parabrachial nucleus and commissural nucleus tractus solitarius, as previously observed in chronic morphine-treated animals. In addition, decreased mu opioid-stimulated [^{35}S]GTPγS binding was found in thalamus and amygdala following heroin self-administration. Despite this decrease in mu-activated G-proteins, [^{3}H]naloxone binding demonstrated increased mu opioid receptor binding in several brain regions following heroin self-administration, and there was significant decrease in mu receptor G-protein efficiency as expressed as a ratio between agonist-activated G-proteins and mu receptor binding. No effects on agonist-stimulated [^{35}S]GTPγS binding were found for any other receptor examined. The effect of chronic heroin self-administration to decrease mu-stimulated [^{35}S]GTPγS binding varied between regions, and was highest in brainstem and lowest in the cortex and striatum.

To examine potential receptor/G-protein mechanisms involved in chronic heroin effects, rats were non-contingently administered saline or increasing doses of heroin i.v. hourly up to 288 mg/kg/day over 40 days. In brain sections, chronic heroin administration decreased DAMGO-stimulated [^{35}S]GTPγS binding in medial thalamus and amygdala, with no effect in cingulate cortex or nucleus accumbens. Chronic heroin administration also reduced [^{35}S]GTPγS

binding stimulated by the principal metabolite of heroin, 6-monoacetylmorphine. In contrast, no significant changes in mu opioid receptor binding were observed in amygdala or thalamus using [^{3}H]DAMGO autoradiography. In membranes from amygdala and thalamus, chronic heroin treatment decreased the maximal effect of DAMGO in stimulating [^{35}S]GTPγS binding, with no effect on DAMGO potency. [^{35}S]GTPγS saturation analysis showed that chronic heroin treatment decreased the B_{max}, and increased the K_D, of DAMGO-stimulated [^{35}S]GTPγS binding.

To examine the time course of mu receptor/G-protein desensitization, rats were treated with saline or heroin for 4, 8, 16, 30 or 40 days, and brain sections were assayed for mu–activated G-proteins by DAMGO–stimulated [^{35}S]GTPγS autoradiography. Hot plate analgesia tests revealed that heroin–treated animals were tolerant at each of these time points. Chronic heroin administration had no significant effect on DAMGO–stimulated [^{35}S]GTPγS binding in any regions after 4 days treatment. Significant decrease in DAMGO-stimulated [^{35}S]GTPγS binding was first observed in parabrachial nuclei after 8 days treatment, in medial thalamus after 16 days treatment, and in amygdala after 30 days treatment. Next, the relationship between recovery of normal mu receptor/G-protein coupling and physical dependence was investigated by examining animals after 12-120 hours of drug abstinence following 30 days chronic heroin administration. Recovery of normal DAMGO–stimulated [^{35}S]GTPγS binding proceeded in reverse order of the time course of uncoupling, with recovery occurring first in caudate/putamen, thalamus and amygdala, with brainstem regions remaining desensitized after 5 days abstinence from heroin when no signs of physical dependence were evident.

These results show that chronic heroin treatment produces significant uncoupling of mu receptors from G-proteins in brain. The regional differences in these effects may help explain why tolerance and dependence develops at different rates to different opioid effects. Indeed, the time course studies suggest that those physiological effects of opioids mediated at the brainstem level may desensitize significantly before those mediated by higher brain structures.

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EFFECTS OF ACUTE AND CHRONIC OPIATES ON SIGNAL TRANSDUCTION EVENTS

F. L. Smith, S. P. Welch, and W. L. Dewey

Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA

The significance of phosphorylation events in acute and chronic opioid exposure has been investigated using primarily *in vitro* methodological approaches. The conclusion of most studies is that signal transduction events are changed in response to the chronic presence of opioid. Behavioral measures have been increasingly used to implicate specific signal transduction events in mediating opioid tolerance and dependence. Recently, Bernstein & Welch (1997) reported that an inhibitor of PKA (KT5720) injected icv. reversed antinociceptive tolerance in morphine pellet-implanted mice. Phospholipid signal transduction systems have also been implicated in opioid tolerance, with most studies focusing on protein kinase C (PKC). For example, PKC inhibitors such as chelerythrine chloride, H7 and calphostin C were able to prevent or reverse acute antinociceptive tolerance to *mu-* and *delta-*opioid agonists (Fundytus & Coderre, 1996; Bilsky *et al.*, 1996; Narita *et al.*, 1995; 1996). Furthermore, tolerance has been associated with changes in the activity and levels of PKC throughout the neuraxis (Mao *et al.*, 1995; Ventayol *et al.*, 1997). Chronic morphine, heroin or methadone administration significantly reduced PKC-*alpha/beta* immunoreactivity in rat cerebral cortex, brainstem and hypothalamus (Ventayol *et al.*, 1997). In opposite fashion, PKC-*gamma* immunoreactivity was significantly increased in spinal cord laminae I and II of morphine tolerant rats (Mao *et al.*, 1995). Studies were conducted in this laboratory to examine the involvement of different

components of the phosphatidylinositol (PtdIns) and phosphatidylcholine (PtdCholine) pathways in morphine tolerance. The hypothesis was tested that central administration of inhibitors of multiple components of phospholipid pathways would lead to an acute reversal of morphine tolerance. Our data indicate that both the PtdIns and PtdCholine pathways contribute to morphine tolerance.

Seventy-two hours after implantation of placebo or 75 mg morphine pellets, mice acutely injected icv. with inhibitor drug (i.e., 30-min before testing) were challenged with morphine s.c. for generation of dose-response curves in the tail-flick test. Placebo pellet-implanted mice received doses of inhibitor drug having no effect on morphine's potency, in order to test for tolerance reversal in morphine pellet-implanted mice. Injection of the phosphatidylinositol-specific phospholipase C inhibitor ET-18-OCH_3 significantly reversed tolerance, indicating a potential role for inositol 1,4,5-trisphosphate (IP_3) and protein kinase C (PKC) in tolerance. Alternatively, phosphatidylcholine-specific phospholipase C increases the production of diacylglycerol and activation of PKC, without concomitant production of IP_3. D609, an inhibitor of phosphatidylserine-specific phospholipase C, also reversed tolerance. Heparin is an IP_3 receptor antagonist, and has been shown to block G protein-coupled receptor kinases (GRKs) (Kunapuli *et al.*, 1994). GRKs regulate the responsiveness of *mu*- and *delta*-opioid receptors through agonist-specific receptor phosphorylation, desensitization and internalization. Low molecular weight (LMW) heparins have been shown to be membrane permeable compared to those used clinically. Injection of LMW heparin (6000 MW) also reversed morphine tolerance in mice. The role for PKC was examined with three structurally dissimilar ATP-binding site drugs that selectively inhibit PKC. Bisindolylmaleimide I, Go-7874, and sangivamycin all significantly reversed morphine tolerance.

Experiments were also conducted to determine the duration of morphine tolerance reversal by two PKC inhibitors. Both Go-7874 and sangivamycin persistently reversed morphine tolerance. Morphine tolerance was reversed 4-, 8- and 24-h after icv. administration of either inhibitor. Therefore, the persistent reversal of tolerance suggests that PKC plays a pivotal role in the long-term maintenance of tolerance. In other words, the inhibition of PKC for 24-h did not lead to a compensatory change in which other protein kinases took the place of PKC to re-establish morphine tolerance. In summary, these studies indicate that chronic morphine exposure leads to changes in phospholipid metabolism that have a direct role in maintaining a state of tolerance.

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THE ROLE OF POTASSIUM CHANNELS IN OPIOID TOLERANCE AND DEPENDENCE

S. P. Welch

Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA

Morphine-induced antinociception and tolerance involves modulation of potassium efflux via K-ATP channels, those potassium channels gated by the ratio of ATP/ADP in cells. Although morphine has been shown to interact with other types of potassium channels, in particular those that are inward-rectifiers and are G-protein coupled (GIRKs), the focus of this presentation is on those inward-rectifying potassium channels which are not classified as GIRKS, but rather are ATP-gated. Morphine induces an efflux of potassium from neuronal cells leading to a state of hyperpolarization via its action at the above variety of potassium channels. Morphine acts to open K-ATP channels allowing potassium to leave the cell. The role of K-ATP channels in the antinociceptive effects of morphine have been verified electrophysiologically, as well as by the use of the K-ATP blocker, glyburide, which attenuates morphine-induced antinociception and precipitates a withdrawal syndrome in morphine-tolerant mice. In addition, tolerance to morphine results in an increased number and decreased affinity of K-ATP binding sites as quantified by ^{3}H-glyburide binding.

Conversely, K-ATP channel openers, such as minoxidil, and levocromakalim, also open K-ATP-gated potassium channels, allow potassium to leave the cell, and produce hyperpolarization. The K-ATP openers produce antinociceptive effects following central administration [the drugs do not pass the blood-brain barrier] and produce antinociceptive effects. We have shown that the K-ATP openers produce antinociception via the interaction with opioid systems. Such work was performed using antagonists to the opioid receptors, antisenses to the opioid receptors and antibodies to opioid receptors. Our work was thus of an indirect type, but clearly indicated that the mu opioid receptor (MOR) and the delta opioid receptor (DOR) were critical to the antinociceptive effects of the K-ATP opener-induced antinociception. Such data led us to hypothesize that the K-ATP openers were releasing endogenous opioids. Opioid antagonists or antisense to opioid receptors would block such release. Such a hypothesis appeared to explain the antinociceptive effects of the K-ATP openers since none of the openers bound to opioid receptors directly. Similarly, the K-ATP openers were not cross-tolerant to morphine, DPDPE, or the kappa agonist, CI-977. The combination of opioids and K-ATP openers will produce greater-than-additive antinociceptive effects that we attributed to the release of endogenous opioids by K-ATP openers. We concluded that the use of a low-dose combination of K-ATP openers with morphine might decrease the development of tolerance to morphine, while maintaining antinociceptive efficacy. In order to more thoroughly address our hypothesis, we quantitated the release of endogenous opioids following the administration of K-ATP openers, morphine, and the drugs in combination.

The K-ATP channel opener, minoxidil, was evaluated for antinociceptive effects in the rat following spinal cord perfusion of the drug in anesthetized, cannulated rats. Cannulation was via the cisterna magna extending to the spinal lumber level via an 8.5 cm cannula of PE10 tubing. A peristaltic pump delivered drug at the rate of 30 microliters/min in a 20 microliter bolus. The ED50 (i.t.) for minoxidil in the rat was determined using the tailflick test [ED50 = 100 micrograms/rat]. Minoxidil-induced antinociception was antagonized in a dose-related manner by the nonspecific opioid blocker, naloxone, and the selective DOR blocker, naltrindole. The kappa opioid blocker, nor-binaltorphimine, failed to alter minoxidil-induced antinociception. In rats administered minoxidil, no release of met-enkephalin, leu-enkephalin, beta-endorphin, or dynorphin A (1-17) was observed as quantified by radioimmunoassay of CSF extracted via reverse peristaltic pumping and flushing of the spinal cavity with artificial CSF. However, a low dose of morphine (1 microgram/rat, i.t.) with no antinociceptive activity, induced the release of both leu-enkephalin and beta-endorphin. In addition, the combination of a low dose of morphine (1 μg/rat) plus minoxidil (12.5 μg/rat) produced antinociception equiefficacious to 100 μg/rat minoxidil alone or 5 μg/rat of morphine alone.

We hypothesize based upon the data described above that endogenous opioid tone is a critical factor in minoxidil-induced antinociception. That is, minoxidil does not release endogenous opioids, but in some manner endogenous opioids act as a "co-agonist" with minoxidil at K-ATP channels. Such data would explain the block of minoxidil-induced antinociception by opioid antagonists in the absence of direct action of minoxidil at opioid receptors. In addition, we have demonstrated that morphine releases endogenous opioids at non-antinociceptive doses of morphine alone. We hypothesize that the release of endogenous opioids by morphine is responsible for the enhancement of minoxidil by morphine. Our data thus strongly indicate that the combination of centrally acting potassium channel openers with morphine might provide a useful efficacious analgesic devoid of the majority of side effects observed with much higher doses of either drug.

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SYMPOSIUM VII

ETHNIC AND CULTURAL ISSUES IN DRUG ABUSE AND DRUG DEPENDENCE RESEARCH

*D. J. Geyen *and B. J. Primm,^ Chairpersons*

***Sam Houston State University, Huntsville, TX**
^Addiction Research Treatment Corps., Brooklyn, NY

INTRODUCTION

The demographics of American society are changing. More and more, people all across the United States are celebrating the uniqueness of their culture and ethnic composition. While many similarities are recognized among various ethnic groups, it is equally important to acknowledge those characteristics that make up the differences. The differences serve as a foundation for cultural norms. Cultural not only influences adaptive and normative behavior, but it also finds expression in drug dependence research. The different cultural norms between groups, are not perceived as deficits among groups. Yet they represent an opportunity for learning, understanding, and creating a heightened awareness for drug dependence research. Therefore, the objectives of this symposium are to: (1) address biomedical and behavioral science drug/alcohol dependence research issues among people of color and women; (2) strengthen the scientific research awareness on ethnic and cultural differences related to drug/alcohol dependence; (3) assist the College on Drug Dependence and the National Institute of Drug Abuse to facilitate more people of color and women to participate, conduct, and lead in drug/alcohol dependence research.

COMPARATIVE RESEARCH ON THE PREVALENCE OF DRUG USE IN HISTORICAL AFRICAN AMERICAN COLLEGES AND NON HISTORICALLY AFRICAN AMERICAN COLLEGES

U. J. O. Baily

Howard University, Washington, D.C.

Historically, African Americans who attend college are less likely to abuse substances than their white counterparts. For example, several national surveys suggest those African American college students at Historically Black Colleges and Universities (HCBUs) drink smaller quantities of alcohol than white students, drink less often each week, and binge drink less frequently than students at majority (white) school. African American students exhibit these patterns of reduced drinking activity regardless of whether they are enrolled at black schools or majority institutions. The negative consequences from alcohol and other drug use are less prevalent among all students on black campuses than majority campuses. White students attending black schools drink less alcohol and binge drinks less often compared to white students at majority schools.

This does not mean that HCBUs do not have alcohol use problems. Rates may vary widely across institutions due to differences in the student body and even between the general characteristics of the school. The recent survey also found that African American males attending public HCBUs had the highest rate of binge drinking overall, while African American males at private HCBUs had the highest rate of lifetime alcohol use. Lack of social support has been identified as a strong predictor of drinking among African American college males. Younger age at first use, parental approval of drinking, numbers of drinking friends and low grade point averages all related to the consumption of alcohol. Another study reported that 19 percent of the population on one HBCU campus was classified as adult children of alcoholics.

THE DRINKING PATTERNS AND ALCOHOL PROBLEMS AMONG WHITES, BLACKS, AND HISPANIC IN THE UNITED STATES

R. Caetano

The University of Texas, Houston, TX

The presentation described trends in drinking patterns and alcohol related problems among whites, blacks, and Hispanics in the United States. To the extent that this is possible, results for Hispanics were presented by national group. Data came from two national samples (1984 and 1995) of whites, blacks, and Hispanics 18 years of age or older living in the continental United States. Analysis of trend data between 1984 and 1995 showed that the rate of abstention has remained stable among whites but increased between blacks and hispanics. Frequent heavy drinking decreased among white men (from 20% to 12%), but remained stable among blacks (15% in both surveys) and Hispanic men (17% and 18%). Results for women were similar to those for men but smaller in magnitude. Additionally, white men and women were two times more likely to be frequent heavy drinkers in 1984 than in 1995. Trend analyses of alcohol problems showed that between 1984 and 1995, alcohol problems were stable between white and black men and increased among hispanic men. The rate of three or more alcohol problems for men of each ethnic group for 1984 and 1995 was: 12% and 11% for white men, 16% and 13% for black men, and 9% and 16% for hispanic men respectively. Problem prevalence was stable and relatively low among women in all three ethnic groups. These data indicated that the prevalence of alcohol problems continued to be high among men in the United States. Even though recent research has shown rates of frequent heavy drinking among white men have declined, this analysis found no corresponding decrease in problem prevalence. Rates of frequent heavy drinking and alcohol related problems between 1984 and 1995 have remained especially high between black and hispanic men suggesting that men of these two ethnic groups should be specifically targeted for renewed prevention efforts.

BARRIERS RELATED TO SUBSTANCE ABUSE RESEARCH AMONG ASIANS

M. Hesslebrock

The University of Connecticut, West Hartford, CN

According to the U.S. Census, by the year 2010, the American population will consist of 71 percent Caucasian, 13 percent Hispanic, 11 percent African American, 4 percent Asian and Pacific Islander, and 1 percent Native American. More specifically it is predicted that number of people with Asian and Pacific Island ethnicity will continue to grow in the United States. The U.S. Census reported the American Asian and Pacific Island population included: 24 percent Chinese, 20 percent Filipino, 12 percent Japanese, 12 percent Indian, 12 percent Korean, 9 percent Vietnamese, 2 percent Cambodian, 2 percent Laotian and 7 percent other. These demographics present implications for drug/alcohol dependence research. Among the implications is the increasing number of Asian and Pacific Island groups living in the United States. There are also a high proportion of foreign-born individuals who speak American English as their second language. In addition, the foreign-born groups may experience immigration and resettlement problems. Furthermore, the problems may include a lack of life support resources, unstable economic development, and inadequate social support systems. The literature has suggested cultural norms, beliefs, and stereotypes unique to the Asian population which present an obstacle to research. For example, there are biological factors such as the ALDHZ genotype, and sociocultural factors that include: downward social mobility, traditional family systems, social isolation, and social adjustment of Asian youth. Suggestions for overcoming barriers and conducting substance abuse research are offered. For example, providing outreach for potential research subjects, following proper protocol, establishing credibility, maintaining legal compliance, and assuring confidentiality. The gender and ethnicity of the researcher are important for breaking the barriers related to substance abuse research among Asians.

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RESEARCH ON THE CULTURAL IMPLICATIONS RELATED TO ALCOHOL CONSUMPTION AMONG AMERICAN INDIANS LIVING ON RESERVATIONS

P. May

The University of New Mexico, Albuquerque, NM

In recent years it has been considered axiomatic in treatment circles that an adherence to traditional American Indian belief, religion, culture were important for Indians seeking to recover from alcohol and drug abuse. This belief has been held in spite of the fact that there is a wide diversity among most tribes regarding adherence to and belief in traditional culture and religion. In this presentation we examined the evidence regarding the effects of traditionality on the use and abuse of alcohol and other substances. The data utilized are from a recently completed random sample of more than 1500 enrolled members of Four Plains Indian Tribes in North Dakota, South Dakota, and Montana who lived on or around their reservations. Various measures of traditionality will be explored as independent variables, and various measures of quantity, frequency, variability and context of use will serve as dependent variables. Male and female differences were emphasized as drinking patterns are highly differentiated by sex.

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SYMPOSIUM VIII

PHENOTYPIC DIFFERENCES IN DRUG EFFECTS RELATED TO BEHAVIORAL TRAITS VERSUS STATES

L. H. Gold and N. E. Goeders, Chairpersons

The aim of this symposium was to highlight studies, across species of laboratory animals, investigating the contribution of trait versus state factors to phenotypic differences associated with drugs of abuse. Rodent models are valuable for investigating traits associated with a vulnerability profile of drug abuse and the impact of recent social stressors. Nonhuman primate colonies afford the opportunity to study drug effects in a social setting, wherein both trait and state influences exert effects. Across models, stress/anxiety, novelty-seeking, and social status are critical variables for conferring differences in phenotypic response to drugs of abuse.

STRESS, THE HYPOTHALAMIC-PITUITARY-ADRENAL AXIS, AND VULNERABILITY TO DRUG ABUSE

N. E. Goeders

Department of Pharmacology and Therapeutics, Louisiana State University Medical Center, Shreveport, LA

The hypothalamo-pituitary-adrenal (HPA) axis consists of a complex, well-regulated interaction between the brain, anterior pituitary gland, and adrenal cortex. During the last several years, our research program has focused on the involvement of stress and the subsequent activation of the HPA axis in cocaine reinforcement. Behaviorally, there are at least three general phases in the etiology of drug self-administration to consider, and knowledge of the interactions between stress and cocaine during each of these is essential for understanding the complex interplay through which stress increases vulnerability to cocaine seeking. These phases include: the acquisition phase, where an animal first comes into contact with cocaine and begins to be aware of its ability to produce reward; the maintenance phase, during which the animal has already learned that the drug is a reinforcer and what responses are required for its subsequent presentation; and the extinction and reinstatement of drug seeking, which is considered as an animal model of relapse. Our research program has investigated the role for the HPA axis in each of these three phases of cocaine self-administration.

In our earlier studies, we investigated the effects of response-contingent (controllable) and response-independent (uncontrollable) electric footshock presentation on the acquisition of intravenous cocaine self-administration in rats (Goeders and Guerin, 1994). We found that exposure to uncontrollable electric footshock shifted the ascending limb of the acquisition dose-response curve upwards and to the left, indicating that these rats were more sensitive to the reinforcing effects of low doses (i.e., 0.125 mg/kg/infusion or lower) of cocaine. This increased sensitivity to cocaine was positively correlated with stress-induced increases in plasma corticosterone, and self-administration did not occur unless plasma corticosterone was increased above a critical level or threshold (Goeders and Guerin, 1996b). Electric footshock did not appear to affect responding maintained by higher doses of cocaine that fell on the descending limb of the dose-response curve, most likely since the cocaine infusions alone were sufficient to increase plasma corticosterone above this critical reinforcement threshold. Therefore, we initiated a new experiment designed to investigate the effects of exogenous injections of corticosterone on the acquisition of cocaine self-administration (Mantsch *et al*, 1998) to determine if the effects of footshock might have resulted from the stress-induced activation of the HPA axis and the subsequent secretion of corticosterone. Similar to what we observed with electric footshock, daily pretreatment with corticosterone (2.0 mg/kg, ip) also produced a leftward shift in the ascending limb of the dose-response curve for the acquisition of self-administration, indicating that corticosterone-treated rats were more sensitive to the reinforcing effects of cocaine. All of the corticosterone-treated rats acquired self-administration at the 0.0625 mg/kg/infusion dose or lower, whereas none of the saline-treated rats acquired this behavior until the 0.125 mg/kg/infusion dose or higher. Since the results from the experiments described above suggest that increasing plasma corticosterone, either through exposure to stress or via exogenous injections of the hormone, can influence the acquisition of cocaine self-administration in rats, the following experiment was designed to further examine the role for the HPA axis in cocaine reinforcement by investigating the effects of adrenalectomy on the acquisition of self-administration (Goeders and Guerin, 1996a). While a typical inverted "U" shaped dose-re-

sponse curve for cocaine self-administration was generated by sham-treated rats, adrenalectomized rats did not learn to self-administer cocaine at any dose tested. These data support the results and conclusions obtained in the experiments described above and suggest that plasma corticosterone may be necessary for the acquisition of cocaine self-administration in rats.

Since corticosterone may be involved in the acquisition of cocaine self-administration, the following experiments were designed to investigate the effects of a reversible "pharmacological adrenalectomy" on the maintenance of this behavior using metyrapone (Goeders and Guerin, 1996a) and ketoconazole (Goeders *et al*, 1998). Pretreatment with metyrapone resulted in significant dose-related decreases in both plasma corticosterone and ongoing cocaine (0.25 mg/kg/infusion) self-administration, suggesting that corticosterone is involved in the maintenance as well as the acquisition of cocaine self-administration. Pretreatment with ketoconazole also reduced low dose (i.e., 0.125 - 0.25 mg/kg/infusion) cocaine self-administration without affecting food-reinforced responding. In fact, pretreatment with ketoconazole resulted in rates and patterns of self-administration at these doses that were indistinguishable from those observed during cocaine extinction. These effects were attenuated, however, when the highest dose of cocaine tested (i.e., 0.5 mg/kg/infusion) was self-administered. This is a critical distinction since the ascending limb of the cocaine dose-response curve is believed to be more involved with the reinforcing effects of the drug, while the descending limb may also be affected by nonspecific rate-decreasing effects resulting from higher doses of the drug. Ketoconazole reduced plasma corticosterone in rats trained with the lower doses of cocaine, but did not significantly affect the hormone when the highest dose was self-administered, suggesting that ketoconazole may have reduced drug-intake, at least in part, through its effects on this stress-related hormone. However, while these experiments demonstrated an important role for corticosterone in cocaine self-administration, cocaine-induced increases in plasma corticosterone ultimately result from the effects of the drug on CRH secretion from the hypothalamus. Therefore, the following experiment was designed to determine the effects of pretreatment with CP-154,526, a centrally active, small molecule CRH receptor antagonist, on intravenous cocaine self-administration in rats (Goeders and Guerin, 2000). Cocaine self-administration was significantly attenuated, and in some cases completely eliminated, following pretreatment with CP-154,526. Drug intake was decreased across all doses of cocaine tested, with the dose-response curve for cocaine self-administration effectively shifted downward and flattened. Furthermore, responding on the cocaine lever following CP-154,526 pretreatment was significantly suppressed, even during the first 15 minutes of the session, a time when rats typically sample the cocaine lever during extinction (Goeders *et al.*, 1998), suggesting that CRH receptors may also be involved in some of the conditioned effects of cocaine as well (Goeders *et al.*, 2000). These data underscore a potential role for CRH in cocaine reinforcement and further suggest a role for the peptide in cocaine addiction and withdrawal.

The role for corticosterone in the electric footshock-induced reinstatement of extinguished cocaine-lever responding was determined by pretreating rats with ketoconazole 30 min prior to electric footshock exposure, which was presented immediately before reinstatement testing (Mantsch and Goeders, 1999). Extinguished responding in vehicle-treated rats was significantly increased following exposure to electric footshock, while no significant increases in responding were observed in ketoconazole-treated animals, suggesting that ketoconazole blocked stimuli associated with the reinstatement of cocaine-seeking behavior. A potential involvement of corticosterone was suggested since ketoconazole pretreatment also significantly decreased the plasma corticosterone response to electric footshock. We have recently investigated the ability of a cue (i.e., a tone and house light) previously paired with cocaine self-administration to reinstate extinguished cocaine-seeking behavior (Goeders *et al.*, 2000). The response-contingent presentation of a light and tone stimulus previously paired with cocaine during self-administration reliably reinstated extinguished cocaine-seeking behavior, while the non-contingent presentation of the same stimulus did not. Although conditioned increases in plasma corticosterone were evident during cocaine extinction as well as during reinstatement, plasma corticosterone returned to basal levels by the end of the session during extinction, while it remained elevated through the end of the session during reinstatement. Pretreatment with either ketoconazole or CP-154,526 reversed the conditioned cue-induced reinstatement of extinguished cocaine-seeking behavior, and ketoconazole also attenuated the conditioned increases in plasma corticosterone observed during reinstatement. These data suggest an important role for CRH and corticosterone in the ability of environmental cues to stimulate cocaine-seeking behavior in rats. The HPA axis may, therefore, also be involved in cocaine craving induced by exposure to cocaine-associated cues in humans.

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ELECTROPHYSIOLOGICAL CORRELATES OF ENHANCED VULNERABILITY TO COCAINE SELF-ADMINISTRATION

M. Marinelli, D. C. Cooper, and F. J. White

Department of Cellular and Molecular Pharmacology, Finch University of Health Sciences/The Chicago Medical School, North Chicago, IL

It is well known that both differences between individuals (individual "traits") and repeated exposure to drugs (drug-induced "states") may participate in determining drug addiction. We have examined the role of individual traits, drug-induces states, and the interaction between the two in determining vulnerability to drugs. Using *in vivo* extracellular recordings, we examined the impulse activity of midbrain dopamine (DA) cells, as these neurons are considered one of the main neurological substrates mediating the reinforcing effects of drugs of abuse.

Individual traits. The concept of inter-individual differences mediating vulnerability to drug addiction started with the observation that there is considerable variation between individuals with respect to sensitivity to addictive drugs. It was hypothesized that certain "individual traits" would make some subjects vulnerable to the addictive properties of drugs, and others resistant. In animals, this individual propensity to develop drug self-administration (SA) can be predicted by drug-independent behavior, such as the level of motor activity during the exposure to a novel environment. Rats with high levels of locomotor activity in a novel environment (High Responders, HRs) show greater reactivity to psychostimulant drugs compared to animals with low levels of motor activity (Low Responders, LRs). Using this HR/LR model, it has been shown that HRs have higher DA levels in the nucleus accumbens compared to LRs. No studies have determined the origins of this difference. In our first experiments, we determined whether differences in DA neuron activity of HRs and LRs could underlie individual differences in vulnerability to drug SA. Animals screened for their response to a novel environment were tested for acquisition of cocaine SA and, in a separate experiment, for the impulse activity of midbrain DA neurons. Only HRs developed cocaine SA (175 μg/kg/infusion) and there was a positive correlation between locomotor response to a novel environment and cocaine intake (fig. 1a). Differences in cocaine SA were not due to differences in sampling because these behaviors did not differ in HRs and LRs self-administering a saline solution. HRs also showed enhanced impulse activity of DA neurons compared to LRs. Both the firing rate (fig. 1b) and the bursting activity (46.0% ± 3.5 vs 32.6% ± 3.3 bursting action potentials; 7.6 ± 0.6 vs. 5.0 ± 0.6 burst events/10 sec) were greater in HRs compared to LRs. In addition, inhibition of firing rate by the DA D2 class receptor agonist quinpirole was attenuated in HRs as compared to LRs, suggesting that increased impulse activity in HRs may, at least in part, be associated with functional sub-sensitivity of impulse-regulating DA autoreceptors.

Drug-induced states. Though vulnerability to drugs may be endogenously present in certain individuals, it may also be induced by exposure to psychostimulants. Repeated drug administration increases behavioral responses to drugs (i.e. behavioral sensitization), and increases drug SA. These heightened drug effects depend on drug-induced neuroadaptations. Concerning the activity of DA neurons, repeated exposure to psychostimulants transiently increases the impulse activity of midbrain DA cells and induces sub-sensitivity of impulse-regulating DA autoreceptors. We have argued that such neuroadaptations in the VTA may be necessary for the initiation of sensitization and increased vulnerability to drug addiction. However, these studies have been performed using non-contingent (experimenter delivered) drug injections; little is known about the effects of voluntary drug exposure. In addition, the consequences of autoreceptor sub-sensitivity on drug craving are unknown. In this second series of experiments, we studied impulse activity of DA VTA neurons following cocaine SA; in parallel, we studied the consequences of autoreceptor activation on drug craving. After stabilization of SA behavior (500µg/kg/infusion, average of 20 mg/kg/day for 7 days), we recorded the activity of DA neurons 1, 3, 10 and 30 days after withdrawal from SA. Rats that self-administered cocaine showed an increase in the firing rate of VTA DA neurons as compared to controls (naïve rats or animals that self-administered saline). This effect was greatest on withdrawal day (WD) 1, and decreased in a time-dependent manner on WD3, WD10 and WD30 (fig. 1c). The greater activity of VTA DA cells on WD1 may be related to decreased sensitivity of impulse-regulating DA autoreceptors, as the WD1 group required higher doses of quinpirole to suppress DA neuron activity compared to control rats. Instead, on WD 10, quinpirole-induced inhibition of firing was similar to that of controls. To determine if the functional activity of impulse-regulating DA autoreceptors could modify drug craving, we studied the effects of autoreceptor activation (by administration of autoreceptor-selective doses of quinpirole) on drug seeking behavior. On WD1 (when DA autoreceptors are sub-sensitive), quinpirole did not modify drug-seeking behavior. But on WD10, (when DA autoreceptors are normo-sensitive), quinpirole decreased drug-seeking behavior. This suggests that autoreceptors, by regulating neuronal activity, could participate in modulating craving and seeking behavior.

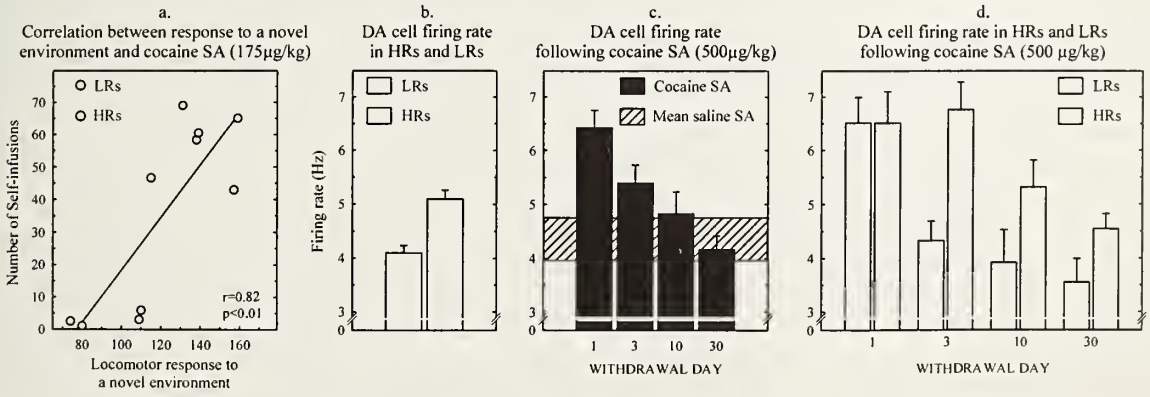

Figure 1. a) There was a positive correlation between response to a novel environment and cocaine SA (average over 7 days). b) HRs had greater firing rate of VTA DA neurons compared to LRs. c) Following cocaine SA, rats showed an increase in DA firing rate on WD1, which returned to baseline values in a time-dependent manner. d) HRs and LRs showed differential neuroadaptations following cocaine SA.

Interaction between individual traits and drug-induced states. The above findings show that increased activity of DA neurons is associated with vulnerability to drugs, either endogenously present in certain individuals (HRs), or induced by repeated exposure to cocaine. In these studies, we tried to determine whether there is an interaction between these two conditions. In this last series of experiments, we studied the activity of DA neurons in HRs and LRs following SA of cocaine (500 µg/kg/infusion for 7 days). In these experiments, HRs and LRs had similar drug intake. In fact, at higher drug doses, both groups of animals acquire SA behavior. Despite similar drug intake, HRs and LRs developed differential neuroadaptations following cocaine SA. Both HRs and LRs showed increased firing rate on WD1, however, LRs showed recovery of DA firing by WD3, whereas HRs maintained increased firing until WD10 (fig. 1d). It is unknown whether this is due to a more efficient normalization process in LRs, or whether it is linked to a system that maintains increased activity in HRs.

Conclusions. Overall, these results show that enhanced impulse activity of DA neurons is associated with increased vulnerability to drugs. In fact, hyperactivity of DA neurons was present in spontaneously vulnerable subjects (HRs), and following voluntary repeated exposure to drugs. This suggests that both "individual traits" and drug-induced "states" converge at the level of DA neurons to modulate vulnerability to drugs. The increased activity of DA

neurons is associated with decreased functional activity of impulse-regulating DA autoreceptors and these changes within the VTA may participate in modulating craving and drug-seeking behavior.

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ANXIOGENIC-LIKE EFFECTS LIMIT REWARDING EFFECTS OF COCAINE IN BALB/CBYJ MICE

L. H. Gold and V. David

Department of Neuropharmacology, The Scripps Research Institute, La Jolla, CA

The reinforcing effects of cocaine can be demonstrated across a wide range of species. However in laboratory studies, not all individual subjects exhibit acquisition of self-administration and similarly not all humans experiencing psychostimulant drugs develop compulsive drug seeking behavior. Recently an increasing number of studies have investigated the biological basis of the vulnerability to drug abuse in outbred rats. A vulnerability profile has been defined based on a number of behavioral, neurochemical and endocrinological features as well as behavioral traits like novelty-induced activity (Hooks *et al.*, 1991; Piazza and Le Moal, 1996). These features are also expressed differentially in inbred strains of mice making such mice a useful model for further examining the relationship between behavioral traits, their underlying biological basis and drug-seeking behavior.

Of particular interest is the BALB/cByJ strain that has not been shown to exhibit cocaine self-administration reliably Deroche *et al.*, 1997; Yu *et al.*, 1997). The ability to perform the necessary operant behavior has been demonstrated in studies of food-maintained responding and self-administration of other abused substances such as heroin and a combination of cocaine and heroin (Deroche *et al.*, 1997; Roberts *et al.*, 1997). These previous results suggested that cocaine may possess properties that interfere with its ability to maintain self-administration in this mouse strain. Our studies explored this issue further using 2 paradigms for cocaine self-administration in BALB/cByJ mice. Using a protocol employing one day for habituation to the test apparatus, BALB/cByJ mice exhibited intravenous cocaine self-administration behavior when using a low dose (0.2 mg/kg/inj) known to be ineffective in several other strains (Carney *et al.*, 1991; Grahame *et al.*, 1995; Deroche *et al.*, 1997; Rocha *et al.*, 1998). However, this initially positive reaction was quickly (5-6 days) followed by a progressive reduction of the number of intravenous cocaine injections. Because we and others have shown that higher acquisition doses are associated with lower rates of self-administration (Deroche *et al.*, 1997; Yu *et al.*, 1997), it is unlikely that the observed reduction in intravenous self-administration was due to the use of a subthreshold dose of cocaine. In fact, higher doses have been shown to produce even greater disruption, with the dose of 1 mg/kg/inj leading to a sudden cessation of responding in BALB/cByJ mice (Deroche *et al.*, 1997). Interestingly, BALB/cByJ mice are more sensitive than other strains to the stimulant effects of lôw doses of cocaine, but dose-related increases in activity have not been observed (Elmer *et al.*, 1996; Deroche *et al.*, 1997). This strain is also exhibits hyper-responsive cellular and behavioral effects to a variety of stressors. Therefore, BALB/cByJ mice may be highly sensitive to both the rewarding and aversive effects of cocaine, and the extinction of self-administration could result from a sensitization of the anxiogenic properties of cocaine. In these mice, diazepam (0.5 mg/kg, i.p.) pretreatment was able to reinstate cocaine-maintained responding. Thus diazepam may have functioned to relieve anxiogenic-like properties of cocaine rather than by enhancing euphoria directly.

Results from an intracranial self-administration study also lend support for a hypothesis of anxiogenic-like effects of self-administered cocaine in Balb/cByJ mice. Mice were trained to self-inject cocaine (10 ng) directly into the NAc in a Y-maze discrimination procedure. Over successive acquisition sessions, these mice made an increasing number of shuttles between the reinforced arm and the start box, this behavior contributing to a significant lengthening of the latency to self-inject cocaine into the NAc. This was unexpected because, in this paradigm, choice of the reinforced arm has been consistently associated with a decrease in the latency to trigger drug injection (David and Cazala 1994; David *et al.*, 1998). Diazepam pretreatment (1.0 mg/kg, ip) in this paradigm reduced the shuttling behavior and concomitantly reduced self-injection latencies. This observation was reminiscent of that reported by Ettenberg and Geist (1991) regarding rats self-administering cocaine intravenously in a runway. These investigators described a form of conflict behavior manifested by an increase in the number of "retreats" from the goal box to the start compartment over successive sessions. The "shuttling behavior" observed in the present study is likely to be the

mouse analog of the "retreat behavior" described in rats. Consistent with this relationship, pretreatment with diazepam reduces both phenomena (Ettenberg and Geist 1991; 1993). Interestingly, site-specific injection of cocaine into the NAc elicited stable self-administration but did not eliminate conflict-like behaviors, suggesting that this region may also mediate anxiogenic or negative affective states.

Emotional reactivity in rodents and humans has been linked with susceptibility to anxiety (Belzung and Berton 1997; Eley and Plomin 1997). Therefore, the finding that an anxiolytic pretreatment helped to maintain cocaine self-administration in an emotional strain of mice has clinical implications and emphasizes the increased addictive potential of mixed drug use. Our results also address the predictive value of vulnerability factors. First, high novelty- or stress-associated emotional reactivity may inhibit stimulant-seeking behavior, because of a necessity to control secondary drug-induced anxiogenic effects. Secondly, sensitivity to the cellular or behavioral effects of stress mediators may also exert a protective action. Thus, the interaction between specific psychological traits (such as emotional reactivity) and the neuropharmacological profile of a given drug (such as cocaine) may be a primary factor contributing to, or preventing, the development of drug use. In these studies, *trait anxiety* (BALB/cByJ) as a genetic factor, and cocaine-induced *state anxiety* as an environmental factor, regulated cocaine self-administration, providing a model predicting the addictive potential of specific genetic-environment interactions.

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SOCIAL STRESS EPISODES: SENSITIZATION AND COCAINE BINGES

K. A. Miczek, H. Covington III, E. M. Nikulina, and T. Kikusui

Departments of Psychology, Psychiatry, Pharmacology, and Neuroscience, Tufts University, Medford, MA

During the last two decades research on the behavioral biology of social stress has benefited from specifying the necessary, sufficient and effective conditions under which episodes of social conflict exert long-term consequences that are evident at the behavioral, physiological and neurochemical level. In addition to the early focus of stress research on ***adaptation***, leading to the proposal of the General Adaptation Syndrome, repeated stress episodes, like intermittent injections with psychomotor stimulants, have been found to engender behavioral and neural ***sensitization.***

At the ***behavioral*** and ***physiological*** level, a *brief* confrontation with a threatening and attacking resident causes an intruder to show defensive and flight reactions, and eventually submissive postures and vocal signals, to express affective distress. Actively coping animals become hyper-defensive after *repeated* brief social confrontations, and when challenged with a psychomotor stimulant, show behavioral sensitization. Intermittent brief social stress episodes differ from continuous subordination stress in that they do not compromise behavioral and physiological activities culminating in morbidity nor in adaptation in terms of tachycardic and hyperthermic responses (Tornatzky and Miczek 1993, 1994).

At the ***pharmacological*** level, intense, unpredictable and uncontrollable social stress episodes engender naltrexone- and naltrindole-reversible analgesia. After repeated social defeat episodes, opiate tolerance and withdrawal, involving *mu* and *delta* receptors become evident (Miczek *et al.*, 1982, Vivian and Miczek, 1999).

Concurrently, episodes of social defeat stress engenders behavioral sensitization to dopaminergic challenges such as by psychomotor stimulants. This sensitization becomes evident several days after a single social defeat stress and, when repeated, lasts for weeks and months (Miczek *et al.,* 1999). Under some conditions, rats that are sensitized by four intermittent episodes of social defeat stress, initiate cocaine self-administration twice as rapidly as controls, maintain drug taking at higher rates, and accumulate cocaine in larger amounts when given continuous access in a "binge" (Miczek and Mutschler, 1996; Tidey and Miczek, 1997; Covington and Miczek, 2000). A most significant feature of the sensitization via intermittent social stress episodes is the emergence of so-called dysregulated cocaine self-administration sooner in the course of a cocaine "binge" than in non-sensitized rats, indicating possibly "loss of control."

Neurochemically, upon confronting a threatening resident rat, dopamine concentrations, as measured via *in vivo* microdialysis, are immediately increased in nucleus accumbens and prefrontal cortex, but not in striatum (Tidey and Miczek, 1996). Repeated social confrontations are anticipated by increased dopamine activity in n. accumbens, and followed by decreased serotonin in this brain area.

Within one hour of the social confrontation, the immediate early gene c-fos is expressed in periaqueductal grey area, dorsal raphe n., and locus coeruleus. In the ventral tegmental area (VTA), but not substantia nigra, the mRNA for mu receptors was doubled after social defeat stress (Nikulina *et al.*, 1998, 1999). *Mu* receptors are located on GABA interneurons that inhibit dopamine neurons in the VTA. In parallel to the behavioral sensitization after repeated social defeat stress, the fos response in the striatum shows evidence for sensitization after amphetamine challenge.

Intermittent, unpredictable episodes of social defeat stress trigger long-term neuroadaptive changes beginning with immediate early gene expression and these changes appear to be long-lasting. These molecular events may be critical for the development of tolerance to opioid analgesia, behavioral sensitization to psychomotor stimulants, and increased stimulant self-administration.

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INFLUENCE OF GROUP SOCIAL BEHAVIOR ON COCAINE'S EFFECTS IN CYNOMOLGUS MONKEYS

M. A. Nader and D. Morgan

Center for the Neurobiological Investigation of Drug Abuse, Department of Physiology and Pharmacology, Wake Forest University School of Medicine, Winston-Salem, NC

The goal of these studies was to achieve a better understanding of the individual differences in susceptibility and vulnerability to the reinforcing effects of cocaine in a nonhuman primate model of drug abuse. Previous research with socially-housed macaques (e.g., Kaplan *et al.*, 1982) has shown that susceptibility to disease states, such as atherosclerosis, reproductive function and viral infection, is influenced by social rank (i.e., dominant vs. subordinate). Differences in these outcome measures across ranks generally have been attributed to the chronic stress associated with being a subordinate monkey. Research in rodents has shown that responses to stressful events can influence individual vulnerability to self-administer stimulants (e.g., Goeders and Guerin, 1994; Piazza *et al.*, 1989). Furthermore, the effects of stress on drug self-administration may be due to stress-induced changes in dopamine (DA) neurotransmission (e.g., Kalivas and Duffy, 1989); DA mediates in large part, the reinforcing effects of cocaine. In the present study, we first examined individually-housed monkeys to determine whether locomotor activity, neuroendocrine function and DA D_2 receptor levels would be predictive of eventual social rank (i.e., trait variables) and then re-evaluated these animals after they had lived in social groups and had the opportunity to self-administer cocaine.

Twenty experimentally naive adult male cynomolgus monkeys (*M. fascicularis*) served as subjects. Each monkey was fitted with a collar and trained to sit in a primate restraint chair (see Morgan and Nader, 2000 for details). For approximately 1.5 years, the monkeys were individually housed. During that time, body weight, serum cortisol and testosterone levels, and locomotor activity in an open field apparatus (3 x 2 x 1.75 m) were examined. In addition, D_2 receptor levels were assessed in all monkeys using the non-invasive imaging procedure of positron emission tomography (PET; see Mach *et al.*, 1997 for details). It was hypothesized that eventual subordinate monkeys would have higher basal cortisol levels and increased locomotor activity scores; it was not clear whether D_2 receptor levels would be predictive of eventual social rank.

Next, the monkeys were placed in social groups of four (i.e. five pens of four monkeys) and social rank was determined based on outcomes of agonistic encounters between animals (see Morgan *et al.*, 2000b). After 3 months of social housing, serum cortisol and testosterone levels were re-assessed and repeat PET scans were conducted in each monkey. Following completion of the PET studies, monkeys were prepared with indwelling intravenous catheters and subcutaneous vascular access ports and trained to self-administer cocaine (0.003-0.1 mg/kg/injection) under a fixed-ratio (FR) schedule during daily 1 hr sessions.

Body weight and locomotor activity while individually housed correlated with eventual social rank. As we had hypothesized, monkeys that were most active in the open field eventually became the most subordinate monkeys when placed in social groups. Neither levels of cortisol or testosterone (see Morgan *et al.*, 2000b) nor dopamine D_2 receptor levels (see Morgan *et al.*, 2000a) predicted evenual social rank.

The largest effect observed after group formation involved D_2 receptor numbers. While there were no differences in D_2 receptor numbers when monkeys were individually housed, following 3 months of social housing, dominant monkeys D_2 receptor binding potential increased by approximately 20%, while the subordinate monkeys receptor levels did not change. When cocaine was made available to these monkeys, the subordinate animals were more sensitive to the reinforcing effects of cocaine compared to the dominant monkeys.

These findings, in a nonhuman primate model, indicate that vulnerability to cocaine abuse is influenced by environmental variables (i.e., state variables). Furthermore, the mechanism for this enhanced vulnerability appears to be related to dopamine receptors and less influenced by neuroendocrine variables, such as cortisol. Clearly, a better understanding of the variables that contribute to enhanced sensitivity to the reinforcing effects of cocaine may lead to better behavioral and/or pharmacological strategies for the treatment and prevention of cocaine abuse.

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SYMPOSIUM IX

SNOW OR ICE: FORECAST FOR THE 21ST CENTURTY

J. C. Anthony and C. Furr-Holden#, Chairpersons*

Johns Hopkins University*, Baltimore, MD

A symposium at CPDD can be said to achieve its purpose when the speakers' presentations engage the audience in a colloquy about important issues that cut across the boundaries of bench or pre-clinical research, clinical studies, and epidemiological investigations with community samples. This symposium included presentations from the bench sciences, the clinical sciences, and epidemiology, in an effort to consolidate what has been learned about psychostimulant drug use, especially cocaine and methamphetamine, and to forecast what might develop during the 21st century elaborations of what we have seen in the later decades of the 20th century.

Each of the symposium participants was invited to prepare a succinct summary of the presented material. Two of the symposium participants chose to do so.

Peter Kalivas accepted the task and offers an important perspective on the interface between pre-clinical and clinical research on psychostimulants. His summary speaks for itself.

Jim Anthony served as discussant, and during the symposium asked the speakers to address several issues concerning history, policy, epidemiology, clinical research, and pre-clinical studies. His contribution lays out some of the issues that deserve attention in new research on cocaine use, methamphetamine use, and the use of other psychostimulant drugs during the 21st century.

PSYCHOSTIMULANT ADDICTION: NEW INFORMATION FROM THE BENCH

P. Kalivas

Department of Physiology and Neuroscience, Medical University of South Carolina, Charleston, SC

Repeated administration of amphetamine-like psychostimulants produces enduring changes in the behavioral response to subsequent drug administration. For example, while the predominant behavioral responses to acute administration are psychomotor stimulation and euphoria, addicted individuals experience cravings for additional drug and paranoia. These changes become permanent characteristics of the behavioral profile of drug action in addicts and reveals that chronic use is producing long-term changes in brain function. The primary goal of bench research has been to use animal and in vitro models to elucidate the changes in brain function. This is being approached from three general perspectives, 1) the molecular site of action, 2) the physiology of neuronal plasticity and 3) the involvement of environmental stimuli in the neuroplastic changes.

Molecular Site of Action. This is perhaps the arena of greatest success in studying addiction to psychostimulants. All of the amphetamine-like psychostimulants bind to the presynaptic transport proteins responsible for removing monoamine transmitters from the synaptic cleft. Although different psychostimulants bind with distinct affinity profiles for dopamine, serotonin and norepinephrine transporters, all have in common affinity for dopamine transporters and the inhibition of dopamine re-uptake appears to be critical for most clinically relevant drug effects. There are two general mechanisms of actions. Drugs such as cocaine and methylphenidate bind to the transporter and prevent dopamine re-uptake, but are not transported into the cytosol. Drugs such as amphetamine, methamphetamine and MDMA act as false substrates and after binding are transported into the presynaptic terminal. Thus, not only will these drugs inhibit dopamine re-uptake, but they also promote the reverse transport of dopamine from the cytosol into the extracellular space. Thus, drugs acting by this latter mechanism more effectively increase extracellular dopamine content.

Given the relatively widespread distribution of dopamine terminals in the brain, the drug-induced increase in dopamine transmission has widespread effects in many brain nuclei and on both pre- and postsynaptic dopamine receptors. However, bench research has focussed on certain nuclei and receptors thought to be especially critical in

drug and natural reward. This has led to the identification of enduring dopamine-dependent changes in brain regions such as the ventral tegmental area (site of dopamine cell bodies), nucleus accumbens and prefrontal cortex. Moreover, in these brain regions it appears that actions on the D1 receptor family and subsequent increases in the adenylate cyclase and PKA signaling pathway may be most critical in establishing the long-term neuroadaptations critical for establishing the behavioral changes associated with psychostimulant addiction.

Physiology of Neuroplasticity. The repeated aphysiological stimulation of D1 receptors produced by psychostimulant abuse results in certain enduring alterations in gene expression that ultimately encode for changes in cell function that may be critical neuroplastic events producing addictive behaviors. Candidate genes are those that manifest enduring increased or decreased expression in response to repeated drug exposure. A variety of genes have been identified, many of which are directly or indirectly associated with excitatory neurotransmission, including Homer1a, Homer 1bc, delta-fosB, GluR1, Gi and preprodynorphin. The fact that drug-induced changes in dopamine transmission results in changes in the expression of genes associated with excitatory transmission reveals that while dopamine transmission may initiate the neuroplastic events, the enduring neuroadaptations are in glutamate transmission. While glutamate transmission is ubiquitous in the brain, projections from cortical regions such as the prefrontal cortex and allocortical regions such as the amygdala have been of particular interest in studying the neurobiology of addiction for two reasons. First, the prefrontal cortex and amygdala provide dense excitatory innervation of dopaminergic neurons and brain regions such as the nucleus accumbens thought critical for drug reward. Second, both of the prefrontal cortex and amygdala are known to be important for various cognitive functions, including working memory and conditioned reward. Thus, drug-induced changes in neurotransmission in these cortical and allocortical structures may in part mediate the well-established regulation of addictive behavior by conditioned environmental stimuli.

Involvement of Environmental Stimuli in Neuroplasticity. The ability of environmental stimuli previously associated with drug use to induce addiction-related behaviors such as drug craving and paranoia has been repeatedly demonstrated in both human addicts and animal models. Brain imaging studies clearly reveal that stimulus-induced craving is associated with increased metabolic activity in the glutamatergic brain regions outlined above thought to sites of drug-induced neuroplasticity, including the prefrontal cortex and amygdala. In contrast, changes in subcortical dopaminergic structures such as the nucleus accumbens are not produced by such environmental challenges. This observation further supports the contention that while psychostimulants stimulate dopamine transmission to initiate neuroplasticity, in addicts the critical enduring neuroadaptations are in excitatory transmission in brain regions associated with learning and memory. In this way, drug addiction resembles neuroadaptive processing induced by more physiological stimuli that initiate behavioral adaptation.

Conclusions. A primary action of psychostimulants in inducing drug addiction is to increase dopamine transmission and dopamine-dependent cell signaling cascades that ultimately produce long-term changes in gene expression. A critical category of genes affected by the pharmacological elevation in dopamine transmission is genes encoding proteins that alter glutamate transmission in the brain. The changes in glutamate transmission occur in projections from brain regions associated with learning and memory such as the prefrontal cortex and amygdala, and offers at least a partial explanation for the capacity of drug associated environmental stimuli to elicit drug craving and paranoia in psychostimulant addicts.

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J. C. Anthony, Co-Chair

Discussion of 'Snow or Ice: Forecast for the 21st Century'

In this symposium, we have heard an overview of recent observations that cut across the multiple levels of NIDA research mission. From the bench sciences, we have new discoveries about sites and mechanisms of action for individual psychostimulant drugs, summarized in an accompanying article by Peter Kalivas (this volume), with some provocative suggestions for the direction of new research on the genes affected by pharmacological elevation in dopamine transmission, especially those encoding proteins that alter glutamate transmission in the brain From the clinical sciences, we heard Richard Rawson summarize the experience, to date, in the treatment of patients with serious patterns of methamphetamine use, associated problems, and the efforts to restore these patients to more adaptive social functioning and reductions in the use of stimulant drugs. His experience highlights some essential important adaptations of conventional drug dependence treatment as administered when patients present with the clinical features of opioid or cocaine dependence. Amelia Arria offered an epidemiological perspective, drawing upon recent results from the National Household Surveys on Drug Abuse, which re-introduced questions about methamphetamine use after a hiatus during the mid-1990s, as well as results from surveys conducted in Micronesia, where methamphetamine outbreaks have been described since the late 1980s.

Discussion of these presentations from the podium and questions from the floor provoked the symposium panelists to consider a range of issues that were touched upon in their presentations. A selection of these issues is summarized here:

- How salient is glutamate in the pathways that lead from initial psychostimulant exposure to later development of cocaine and methamphetamine dependence syndromes that achieve clinical significance?
- In the experience of clinical practitioners, is methamphetamine use riding on the back of alcohol problems that pre-dated the methamphetamine problems? Is comorbidity of methamphetamine and alcohol use a serious consideration in the treatment of recent methamphetamine users?
- Do the epidemiological data suggest a still-growing outbreak of methamphetamine use, or has there been a series of localized outbreaks that never have gained a national toehold?
- In several countries, less developed than the U.S., would it be wise to increase the social sanctions, including formal punishments, against psychostimulant drug use, along the lines of what has been described for post WWII Japan and China? Have the outbreaks progressed beyond the point at which these draconian measures might be expected to have their greatest impact?
- Why has there been no more explosive spread of methamphetamine (and crack-cocaine use) throughout all the communities of the U.S. of any size?

To the extent that these questions and related questions remain unanswered, there is good reason to sustain the international communication and collaboration that has helped us perceive the variation in population experience with individual drugs. Connections with earlier US epidemics of psychostimulant drug use are clear, and the task for scientists now is to identify the characteristics that perpetuate drug dependence, and then to focus on characteristics that might be influenced in order to reduce the occurrence of drug dependence.

SYMPOSIUM X

ARE THE NEUROIMMUNE CONNECTIONS RELATED TO DRUG ABUSE IMPORTANT IN HUMANS?

T. K. Eisenstein and F. Renaud, Chairpersons

There are a number of papers in the literature documenting alterations in immune function or increased infection in intravenous drug abusers (Brown *et al.* 1974). Addiction can be accompanied by profound alterations in life-style that may independently affect immune responses and resistance to infection, including residence in overcrowded, unsanitary accommodations, poor nutrition, and sharing of needles as a vector for transmission of infectious agents, making it difficult to assess the effects of the drugs themselves on the immune system. A substantial literature has accumulated documenting effects of opioids on immune responses and resistance to infection using animal models. Effects on several different cells of the immune system have been reported, including on rat natural killer (NK) cells (Weber and Pert 1989), rat T-cell responses (Bayer *et al.* 1990), antibody formation by mouse spleen cells (Bussiere *et al.* 1991), and induction of apoptosis or programmed cell death in rat peritoneal and spleen cells (Singhal *et al.* 1997) and in mouse spleen cells (Yin *et al.* 1999). Most of these studies conclude that morphine is immunosuppressive, with the implication that heroin abuse in humans may lead to similar adverse effects on the immune system. There are a few studies using human cells or in human volunteers that parallel the studies in rodents, indicating that the rodent studies are highly relevant. Yeager *et al.* infused morphine into human volunteers for 24 hours and showed definitively that the opioid inhibited natural killer cell activity, and this depression lasted for 8 days (Yeager *et al.,* 1995). The dose used was an initial intravenous loading dose of 0.05 mg/kg followed by continuous infusion of 0.03 mg/kg/hr, with a resultant blood level of 18.5 ng/ml. The results show a striking parallel to those reported by Weber and Pert in rats given morphine into the periaqueductal gray region of the brain, which induced a reduction in NK activity in the spleen. In ex vivo studies, Nair *et al.* (1997), Singhal *et al.* (1999) and Yin *et al.* (1999) all have reported that morphine added to human peripheral blood lymphocytes in doses of 10-6 to 10-8 induces apoptosis or primes for induction of apoptosis. Similar effects were reported in regard to apoptotic effects of morphine for rodent cells by Singhal *et al.* (1997) and by Yin *et al.* (1999). Finally, a recent paper from Thailand followed immune parameters in pure heroin addicts that underwent abrupt withdrawal (Govitrapong *et al.* 1998). They showed depressed responses to a T-cell mitogen that persisted for five days, and alterations in CD4/CD8 ratios that were evident up to 24 months post heroin withdrawal. These results and those of Yeager show that drug abuse in its various aspects can profoundly alter human immune function in a controlled experimental design.

In this symposium, the participants address several new, important aspects of opioid modulation of immune responses. First, Drs. Carrigan and Lysle compare effects of morphine and heroin on several parameters of immune function. These studies are important because of the work from Dr. Pasternak's laboratory indicating that there may be differences in metabolites of these two opioids that can trigger different receptors. Dr. Eisenstein presents data on effects of abrupt versus precipitated withdrawal in mice on capacity to mount a primary antibody response. These studies show a parallel with those of Govitrapong carried out in humans. Dr. Renaud presents an in vitro model system of tolerance and withdrawal using rodent cells, which complements the in vivo model used by Dr. Eisenstein. Dr. Chang examines the effect of morphine on endothelial function and how it may affect leukocyte adherence and permeability of the vasculature to HIV. Finally, Dr. Ho presents data showing that morphine alteration of immune cell function is part of a larger neuroimmune network, as a combination of morphine plus Substance P enhanced HIV infection of human lymphoid cells.

EFFECTS OF HEROIN ON THE IMMUNE SYSTEM

K. A. Carrigan and D. T. Lysle

University of North Carolina at Chapel Hill, Chapel Hill, NC

Morphine administration is associated with alterations in a number of immune parameters (e.g., Bayer *et al.* 1990; Shavit *et al.* 1986; Taub *et al.* 1991). For example, studies in our laboratory have shown that acute morphine treatment in rats suppresses splenic lymphocyte proliferative responses to both T- and B-cell mitogens, splenic

natural-killer cell activity, blood lymphocyte mitogenic responsiveness to T-cell mitogens, and the production of the cytokines interleukin-2 and interferon-γ (Fecho *et al.* 1993; Lysle *et al.,* 1993). Furthermore, the immune alterations induced by morphine are dose-dependent and antagonized by naltrexone, an opioid-receptor antagonist, indicating that the effects are mediated via opioid receptors (Lysle *et al,.* 1993).

In contrast to the wealth of information available regarding the immunomodulatory effects of morphine, little is known about immunomodulatory effects of diacetylmorphine (heroin), a semisynthetic derivative of morphine known for its high abuse potential. As early as the mid-20th century, clinicians noted an increased incidence of bacterial, viral, and fungal infections amongst regular users of heroin (Luttgens 1949; Hussey and Katz 1950). The immune status of heroin users has been shown to be altered (Brown *et al.,* 1974), but the conclusions that can be drawn from those studies about the causal relations is limited. To our knowledge, only one study has directly evaluated the impact of heroin on immune status (Thomas *et al.,* 1995). The results of that study showed that in-vitro exposure of murine immune cells to heroin induces alterations in several immune assays, however those effects were not consistent across assays or dose-dependent.

Heroin is distinct from morphine in a number of ways. Heroin has a more rapid onset of action in comparison to morphine (Scott and Orr, 1969). Differences in potency between heroin and morphine also are well-documented. For example, heroin has been found to be about 16 times more potent than morphine in producing reinforcing effects in animals (Harrigan and Downs 1978; Van Ree *et al.,* 1978) and subjective effects in humans (Seevers and Pfeiffer 1936; Martin and Fraser 1961; Jasinski and Nutt 1972; Kaiko *et al.,* 1981). Similar potency relations have been obtained in a number of different analgesic assays using animals (Shemano and Wendel, 1964; Switzman *et al.,* 1981; Umans and Inturrisi, 1981; Tasker and Nakatsu 1984) and humans (Seevers and Pfeiffer, 1936; Reichle *et al.,* 1962; Dundee *et al.,* 1966; Kaiko *et al.,* 1981). In addition, several reports indicate that animals made tolerant to morphine do not show cross-tolerance to heroin, suggesting that heroin acts through different neurobiological mechanisms than morphine (Bolger *et al.,* 1988; Lange *et al.,* 1980; Rossi *et al.,* 1996). Interesting recent work shows that 3-methoxynaltrexone selectively antagonizes the analgesic actions of heroin, but does not interfere with morphine-induced analgesia (Brown *et al.,* 1997). Collectively, these investigations point out important differences between heroin and morphine and suggest that studies on morphine's immunomodulatory effects might not generalize to heroin.

Given the major health concerns surrounding the abuse of heroin, an understanding of the immunologic consequences of heroin use is critically needed. The present studies begin to address critical gaps in our knowledge about this opioid drug of abuse. In an initial study, rats received either a subcutaneous injection of saline or heroin at doses of .01, .1, 1.0, or 10 mg/kg (Fecho *et al.* 2000). One hour after the injection, the rats were sacrificed and immunological assessments were conducted. The results showed that heroin induces a dose-dependent suppression of concanavalin A-stimulated proliferation of T cells, lipopolysaccharide-stimulated proliferation of B cells, production of interferon, and cytotoxicity of natural killer (NK) cells in the spleen. Heroin was approximately 10 times more potent than morphine in producing functional alterations of immune status. Follow-up investigations showed that the heroin-induced immune alterations are blocked by prior administration of the opioid receptor antagonist, naltrexone. These findings demonstrate that heroin administration induces opioid receptor mediated effects on the immune system as assessed by *ex vivo* measures of immune status.

These findings showing that heroin induces pronounced alerations of immune status build upon the prior work with morphine. However, to more fully understand the immunological consequences of opiates, it is important to assess whether metabolites common to heroin and morphine, such as morphine-6β-glucuronide, produce immunomodulatory effects. Some of our most recent studies have investigated whether administration of morphine-6β-glucuronide induces immune alterations. In the initial study, morphine-6β-glucuronide was given at a s.c. dose of 1.0, 3.16, or 10 mg/kg one hour prior to immunological assessments. The results showed that morphine-6β-glucuronide induces a reduction in a number of immune measures, including proliferation of T- and B-lymphocytes to mitogen, interferon-γ production, and natural-killer cell activity. Subsequent studies showed that i.c.v. administration of morphine-6β-glucuronide produces immunomodulatory effects at doses about 3000 times less than the doses required to produce effects when the drug was administered systemically. Comparing across studies morphine-6β-glucuronide is about 10-fold more potent than morphine in producing immunomodulatory effects when this compound is administered i.c.v. in the rat (Lysle *et al.,* 1996). Given that morphine-6β-glucuronide does not readily cross the blood-brain barrier, the large potency difference between i.c.v. and systemic administration indicates that the immunomodulatory effects of morphine-6β-glucuronide are mediated through the central nervous

system. These results are consistent with our prior work indicating that the effects of morphine are mediated by the central nervous system.

Collectively, the present studies have shown that heroin induces immunomodulatory effects that may contribute to the immunological abnormalities observed in heroin users. Moreover, the present findings indicate that metabolites of heroin and morphine may contribute to the immune aterations induced by those compounds. In general, these findings further establish the direct relationship between opiate use and immune alterations.

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WITHDRAWAL IN MORPHINE-TOLERANT MICE INDUCES IMMUNOSUPPRESSION

T. K. Eisenstein and R. Rahim

Center for Substance Abuse Research and Department of Microbiology and Immunology, Temple University School of Medicine, Philadelphia, PA

Previous work from our laboratory demonstrated that mice implanted with a slow-release 75 mg morphine pellet showed profound immunosuppression as measured by the capacity of spleen cells to mount an ex vivo antibody response to the test antigen, sheep red blood cells (Bussiere *et al.,* 1991). Examination of the time course of the immunosuppression showed that it was maximal at 48 hr post pellet implantation and that by 120 hr immune responses had returned to normal levels (Bussiere *et al.,* 1993). These effects were opioid receptor mediated as they were reversed by naloxone. The present studies investigated the effect of withdrawal from morphine on splenic immune responses. Two different abstinence paradigms were tested: abrupt withdrawal, in which the morphine pellets were removed, and precipitated withdrawal, in which the pellets were removed and mice were given naloxone by subcutaneous injections or by osmotic mini-pumps. Responses of animals undergoing withdrawal were compared to responses of mice which retained the morphine pellet and to placebo-pelleted mice which underwent surgery and had their pellets removed. Some of these animals received saline and some received naloxone as controls for precipitated withdrawal. The dependent state was confirmed by measuring jumping and weight loss in animals undergoing withdrawal. It was found that by 24 hr and 48 after withdrawal, using either paradigm, mice were profoundly immunosuppressed. However, at three hr post precipitated withdrawal a small, but statistically significant, immunopotentiation was observed. By 6 hr, the animals were immunosuppressed. In contrast, mice subjected to abrupt withdrawal showed steadily increasing immunosuppression over the first six hr, with no evidence of immunopotentiation. At time points beyond 48 hrs, the kinetics of the immunosuppression curves for the two paradigms also differed. Animals undergoing precipitated withdrawal had normal immune responses by 72 hrs after initiation of abstinence, whereas mice undergoing abrupt withdrawal were still greater than 50% suppressed at the 144 hr time point. While alterations in spleen weights were observed in animals subjected to both withdrawal paradigms, spleen weight did not correlate with immune responses. At 72 hr, mice in precipitated withdrawal had normal immune responses but still manifested decreased spleen size. Flow cytometry analysis at 24 hr after either abrupt or precipitated withdrawal showed a decrease in the number of macrophages and B-cells in the spleen, and a moderate but not statistically significant increase in the number of T-cells. Previous analysis of alterations in cell subsets done 48 hr after morphine pellet implantation had shown similar alterations in the proportion of subsets of cells (Hilburger *et al.,* 1997). Further experiments were undertaken to examine whether withdrawal resulted in a decrease in function of lymphocytes or macrophages which could account for the immunosuppression. Spleen cells were obtained from mice 24 hr after abrupt or precipitated withdrawal. They were placed in culture with spleen cell fractions from normal mice. The cell fractions were either plastic adherent cells, which are macrophage enriched, or plastic nonadherent, which contain mainly lymphocytes, including B- and T-cells. It was found in preliminary experiments that the macrophage-rich fraction restored the capacity of the spleen cells to make an antibody response to sheep red blood cells, but the nonadherent fraction did not. Although macrophage-rich fractions were effective in reversing immunosuppression for cells of mice in either abrupt or precipitated withdrawal, the effect was more robust for abrupt withdrawal. The observation that a macrophage-rich fraction could restore immunological responsiveness to spleen cells taken from mice undergoing withdrawal from morphine parallels results obtained previously. In the previous studies, a macrophage-rich cell fraction restored responses of spleen cells obtained from mice which had received morphine pellets for only 48 hrs (Bussiere *et al.,* 1993). Studies are in progress to more

definitively establish the nature of the immunologic defect in spleen cells of mice undergoing withdrawal, and to compare the immunological defects in mice undergoing abrupt versus precipitated withdrawal.

These results show that withdrawal causes profound effects on the immune system with immunosuppression being the dominant response. However, comparison of effects on immune responses of abrupt versus precipitated withdrawal also indicates that the withdrawal paradigm affects the immune system in different ways. Exploration of the mechanisms by which withdrawal alters immune responses can be expected to elucidate fundamental connections between the opioid and immune systems. Further, these studies suggest that addicts in withdrawal may be more susceptible to infection, due to suppressed immune responses.

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EFFECT OF MORPHINE ON Fc-MEDIATED PHAGOCYTOSIS BY MURINE MACROPHAGES: A MODEL SYSTEM FOR STUDIES ON TOLERANCE AND DEPENDENCE AT THE CELLULAR LEVEL

F. L. Renaud

Biology Department, University of Puerto Rico, Rio Piedras Campus

It is well known that drug addicts show a propensity to infections, which has been ascribed to the sharing of dirty needles and a strenuous lifestyle (Tubaro *et al.,* 1987). However, recent evidence suggests that opiates may have both direct (Hernandez *et al.,* 1993) and indirect effects (Mellon and Bayer, 1998) on immune cells, which may contribute to weakening of the immune system. We have focused our work on the effect of morphine on phagocytosis by murine macrophages, a key cell in both acquired and innate immunity. Previous work from our laboratory had shown that acute morphine inhibits phagocytosis in thioglycollate-elicited peritoneal macrophages by a naloxone-reversible, dose-dependent mechanism (Casellas *et al.,* 1991; Tomei and Renaud, 1997). Furthermore, chronic morphine seems to induce a putative tolerant/dependent state in these cells, since morphine will not inhibit phagocytosis under these conditions, and drug withdrawal from putatively tolerant cells will result in inhibition of phagocytosis. In this work we study the development of the tolerant/dependent state in greater detail, including some cellular mechanisms that may be involved in this development. Withdrawal-induced inhibition of phagocytosis (WIP) in cells chronically exposed to morphine is shown to take place maximally one hour after thorough washing of the opiate with fresh medium; similar results are obtained when morphine is displaced by naloxone without changing the medium. However, this decrease in phagocytic capability of the cells is time-reversible, and re-addition of morphine several hours after withdrawal will again result in inhibition of phagocytosis by the opiate, as in acute exposure (Lazaro *et al.* 2000). The receptor-specific opioid agonists DAMGO (mu) and U50,488 (kappa) give similar results; namely chronic exposure results in lack of inhibitory effect on phagocytosis, and withdrawal by the specific antagonist (CTOP, mu; Nor-BNI, kappa) results in a transient reduction of phagocytic capability of cells. On the other hand, opioid peptides (endomorphin-1, met- and leu-enkephalin and dynorphin 1-13), although inhibitory in acute exposures, did not result in development of putative tolerance upon chronic exposure. It is also of interest that, although acute methadone does not affect phagocytosis at any concentration tested, it will prevent WIP if it is present in the medium after withdrawal from putatively tolerant cells. The cellular mechanism involved in morphine effects on phagocytosis by macrophages bears a strong similarity to opioid mechanisms in the nervous system. We have evidence that the mu receptor is very important in these effects. This part of the work was performed in collaboration with Drs. Sabita Roy and Horace Loh, from the University of Minnesota, where a mu receptor knockout mouse (MORKO) was developed (Loh *et al.,* 1998). Acute morphine will inhibit phagocytosis by wild type macrophages in this strain of mice, with no effect on cells from MORKO mice. Similarly, chronic morphine will result in WIP in wild type cells, but again withdrawal has absolutely no effect on phagocytosis by chronically treated macrophages from MORKO mice. In terms of signal transduction, Gi proteins appear to be important in effects of both acute and chronic morphine as pertussis toxin (PTX) pre-treatment of macrophages will block inhibitory effects of acute morphine. Similarly, no WIP is observed when PTX-treated cells are exposed chronically to morphine. Cyclic AMP increases have been implicated in some of the withdrawal effects observed in tolerant cells from the nervous system (Nestler 1992; Avidor-Reiss *et al.* 1995), and we have evidence for a similar role for cAMP in macrophages. Drug removal from chronically exposed cells, by either washing or naloxone displacement, will result in a three-fold increase in the level of cAMP. We surmise that the increase in this nucleotide is possibly related to WIP, since an artificial increase in cAMP levels by dibutyrl cAMP in chronically

exposed cells that remain in constant morphine, results in an inhibition of phagocytosis of the same magnitude as that caused by drug withdrawal. Furthermore, agents that inhibit the activity of either adenylate cyclase (dideoxyadenosine) or protein kinase A (HA1004) will prevent WIP in chronically treated cells. These observations strengthen our contention that the increase in cAMP level and inhibition of phagocytosis are causally related. Results obtained with HIV-1 infected cells have been interpreted similarly (Thomas *et al.,* 1997). We have found results with resident cells similar to those reported above with thioglycollate-elicited cells. The phagocytic capability of resident cells increases with culture time, reaching a maximal level after three days in culture. Acute morphine results in a dose-dependent, sustained inhibition of phagocytosis by resident cells. However, morphine is effective at lower concentrations in three-day old cultures, suggesting an increase in receptor affinity or in receptor number. Furthermore, upon chronic exposure, phagocytosis by freshly cultured cells becomes insensitive to morphine, suggesting putative tolerance. On the other hand, phagocytosis by cells from three-day-old cultures is still inhibited by the drug after chronic exposure, although to a lesser degree when compared to acute exposure. These data suggest that state of activation is important in determining the response to morphine by macrophages.

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MORPHINE AFFECTS THE NEURO-IMMUNE AXIS AND ENHANCES PERMEABILITY ACROSS VASCULAR ENDOTHELIAL CELL BARRIERS

S. L. Chang

Department of Biology, Seton Hall University, South Orange, NJ

An immune challenge can affect both the nervous and immune systems. Mediated via the hypothalamic-pituitary adrenal (HPA) axis, a stimulatory effect on the nervous system can be translated into an inhibitory effect on the immune system (Chang *et al.,* 1996). In this way, the induction and progression of immune responses is highly regulated. Leukocyte-endothelial adhesion (LEA) within the post-capillary bed venule is the process by which circulating leukocytes leave the central column of blood cells within the blood vessels and assume a position in contact with the endothelial cell lining. LEA is the prelude to a series of cellular events leading to cellular transmigration across the endothelium. It is, therefore, an indicator of a functional immune response (House and Lipowsky 1987).

Lipopolysaccharide (LPS) is a glycolipid isolated from the bacterial outer membrane. It is a potent endotoxin leading to an acute inflammatory response that can progress to endotoxic shock. The actions of LPS are mediated via various pro-inflammatory cytokines, including TNF-α, IL-1β, and IL-6. In response to systemic administration of LPS, these cytokines are secreted into the plasma. An immune response can result from either systemic treatment with LPS, or direct exposure to these cytokines.

The basal level of LEA is similar in adrenalectomized (ADX) rats and in rats undergoing sham (SHAM) surgical procedures. However, we found that in response to a topical suffusion of the chemotactic peptide, n-formylmethionine-leucyl-phenylalanine (FMLP) at 10^{-7} M, LEA in the ADX rats was significantly greater than that in the SHAM control animals ($p<0.05$), and the percentage increase in LEA in response to FMLP was also significantly greater in ADX rats ($p<0.05$). Following systemic treatment with LPS (100 μg/kg), the SHAM rats exhibited a normal LEA response, as expected. There was little LEA response in the ADX animals. However, the ADX animals did experience disseminated intravascular coagulation (DIC), an indication of endotoxin shock, following the small dose of LPS mentioned above. Although abolishment of glucocorticoid production by the HPA axis following an adrenalectomy does not change the basal level of LEA, it does lead to a potentiation of the LEA response to topical suffusion of FMLP, and an increased incidence of endotoxin shock in response to a systemic challenge by LPS.

Modulation of the HPA axis by exogenous factors, such as morphine, could represent one mechanism underlying the effects of opiate addiction on the brain-immune-axis (Chang *et al.,* 1996). Injection of IL-1β (i.c.v.) significantly increased plasma corticosterone levels of rats given a placebo, but did not increase the corticosterone levels in morphine-tolerant rats. In a parallel study, chronic exposure to morphine resulted in DIC following an i.p. injection

of LPS (250 μg/kg) which is similar to that seen in the ADX animals mentioned above. Therefore, chronic exposure to morphine appears to desensitize the HPA axis to a challenge with IL-1β, and may be related to the development of endotoxin shock seen in morphine tolerant rats, which may have a similar mechanism to that underlying the development of endotoxin shock seen in the ADX animals. Another parallel study showed that chronic exposure to morphine potentiates the production of cytokines including TNF-α, IL-1β, and IL-6 in response to an i.p. injection of LPS (250 μg/kg). These cytokines, in turn, increase leukocyte adhesion to the endothelium, and also increase the permeability of the endothelium, which could allow invasion of pathogens, including HIV-1, across the vascular endothelial barrier (Fiala *et al.,* 1997).

The vascular endothelium, characterized by its impermeable interendothelial tight junctions, provides a crucial interface between the circulating blood and the underlying tissue. Recently, we investigated how morphine affects the integrity of the vascular endothelial cell (VEC) barrier using the VEC barrier model of human coronary artery endothelial cells (hCAEC), rat brain microvascular endothelial cells (rBMVEC), or human brain microvascular endothelial cells (hBMVEC) (Fiala *et al.,* 1997).

Morphine decreased cell viability in a dose-dependent manner in all three endothelial cell types. This effect was not naloxone reversible. LPS also decreased endothelial cell viability with these three cell types, and pre-treatment with morphine enhanced the LPS-induced decrease in cell viability. We also tested if apoptosis may one of the mechanisms underlying the morphine-induced decrease of cell viability using 4'-6-diamidino-2-phenylindole (DAPI) staining. Endothelial cells normally contain a round nucleus. However, some of the morphine-treated endothelial cells contained condensed nuclei, and when co-treated with morphine and LPS, the nuclei of the cells were condensed and irregular. The results from our studies showed that: (1) morphine increased the percentage of apoptotic nuclei in all three endothelial cells in a dose-dependent manner; (2) this effect was not naloxone reversible; and (3) pre-treatment with morphine enhanced LPS-induced apoptosis.

Based on these observations, we hypothesized that morphine modulates the permeability of the VEC barrier to small molecules such as HIV-1. To test our hypothesis, we examined the effects of morphine on the VEC barrier permeability. Each of the three endothelial cell types was used to construct an in vitro VEC barrier model by allowing cells to grow on the upper chamber of an insert on a microporous membrane. This insert was set inside the well of a 24-well cell culture plate. Inulin is a commonly used marker for paracellular transport across endothelial cell barriers. In all three cells tested, morphine increased [^{14}C]-inulin permeability across the VEC barriers in a dose-dependent manner. As seen previously, naloxone did not reverse this effect. In addition, pre-treatment with morphine enhanced the LPS-induced increase in permeability of the VEC barrier. Furthermore, using the Amplicor HIV-1 Monitor Test developed by Roche Laboratories, we showed that HIV penetration across the VEC barrier following exposure to HIV for 24 hr was further enhanced by morphine in a dose-dependent manner. These data support our hypothesis that morphine can enhance viral penetration across vascular endothelial cell barriers which may be relevant to brain dementia.

In summary, we have shown that chronic exposure to morphine in vivo desensitized the HPA axis and potentiated LEA in response to FMLP and IL-1β. Chronic morphine exposure also seemed to sensitize the animal to develop endotoxic shock and to potentiate the production of cytokines, including IL-1β and TNF-α in response to an LPS challenge. These cytokines can enhance the LEA as well as the penetration of HIV-1 across an endothelial barrier. Based on these observations in vivo, we performed in vitro studies and showed that morphine directly decreases the viability of VEC, and potentiates the effects of LPS on cell viability via an apoptotic mechanism. Morphine increased [^{14}C]-inulin permeability across a VEC barrier model, and enhanced the LPS-induced increase permeability to [^{14}C]-inulin, which was naloxone irreversible. Taken together, these results may help to explain some of the mechanisms by which morphine enhances HIV-1 penetration across vascular endothelial cell barriers, including the blood-brain barrier.

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OPIOIDS, SUBSTANCE P AND HIV

W. Z. Ho

The Children's Hospital of Philadelphia, University of Pennsylvania School of Medicine, Philadelphia, PA

Intravenous substance abuse, including opiate addiction is a major risk factor for AIDS. Opiate and other drugs of abuse, such as cocaine, have been implicated as co-factors in HIV infection and progression to AIDS. Opioids modulate functions of the immune system, supporting the existence of a complex, bidirectional link between the CNS and immune system. Although the role of morphine in modulation of immune system and HIV infection of human immune cells has been extensively studied, the precise cellular mechanisms are largely unknown. Evidence accumulated from both *in vitro* and *in vivo* studies indicates that there is an important relationship between morphine and the neuropeptide substance P (SP). Several studies have reported that SP is involved in the pathogenesis of opiate withdrawal. The goal of our research is to understand the mechanism of interaction of opioids (such as morphine, methadone) and SP in the immunopathogenesis of HIV infection and AIDS. Our overall hypothesis is that opioids affect HIV infection of human immune cells by regulating neuropeptides such as SP, and that opioids and SP have a synergistic effect on HIV replication in the immune cells.

SP, IMMUNE CELLS AND HIV

SP has been described almost exclusively as a peptide of neuronal origin. However, in our most recent work, we have made the unique observation demonstrating that human monocytes/macrophages and lymphocytes isolated from human peripheral blood synthesize both SP mRNA and SP protein (Ho *et al.,* 1997; Lai *et al.,* 1998). We have also demonstrated that isolated microglial cells from human brain express SP (Lai *et al.,* Neuroscience, submitted). In addition, we have identified the presence of SP receptor mRNA in these immune cells. SP is a potent modulator of neuroimmunoregulation. SP stimulates human monocytes/macrophages to produce inflammatory cytokines such as interleukin 1 (IL-1) and IL-6 and tumor necrosis factor alpha (TNF-α) (Ho *et al.,* 1998; Ho *et al.,* 1996). These cytokines induce HIV replication in human monocytes and T-lymphocytes. Based on these observations, we investigated the effects of SP on HIV replication in latently infected immune cells. We demonstrated that SP significantly enhanced HIV expression in the chronically infected promonocytic (U1) and T lymphocytic (ACH-2) cell lines stimulated with TNF-α plus PMA. This stimulatory effect of SP was associated with activation of the HIV promoter LTR. Further, the addition of SP to the cultures of latently infected PBMCs isolated from HIV-infected patients enhanced HIV gag gene expression, suggesting that SP may play a potentially important role as a positive regulator of HIV replication in latently infected immune cells. Interestingly, we observed that the SP receptor antagonists CP-96,345 and RP-67,580 not only blocked SP-induced HIV replication in U1 and ACH-2 cells but also potently inhibited HIV R5 strain infection of human macrophages. This inhibitory effect of the SP antagonists was correlated with down-regulation of CCR-5, an HIV entry coreceptor, on human macrophages, indicating that the SP receptor is biologically involved in HIV infection of these cells. By blocking the SP receptor on macrophages, SP antagonists may offer therapeutic potential for patients with HIV infection or AIDS. Collectively, our findings that SP is secreted by human immune cells, participates in immunoregulation of immune cells, and that it up-regulates HIV expression in latently infected immune cells may be of importance in understanding the pathogenesis of immune-mediated events, including neuroimmunologic diseases and AIDS.

MORPHINE AND SP INTERACTION

The effects of morphine on neuropeptides such as SP are of particular interest because both are involved in modulation of the immune system and HIV infection. We have demonstrated that morphine, when added to human immune cell cultures (monocytes, macrophages and peripheral blood lymphocytes), up-regulates SP mRNA expression and SP peptide synthesis. Morphine also induces expression of neurokinin-1 receptor (NK-1R), a primary receptor for SP, in these immune cells. The opioid receptor antagonist (naltrexone) blocks morphine-induced SP expression in human immune cells, supporting the concept of authentic morphine receptor-mediated regulation. Based on these observations, we hypothesize that morphine and/or SP may play an important role in HIV infected drug abusers by affecting functions of monocytes and macrophages, which may prompt HIV replication in infected cells, thus triggering further the disease progression and exacerbate immune deficiency. To test this hypothesis we examined the effect of morphine and/or SP on cytokine production from monocytes/macrophages as well as on HIV replication in these cells. We have made the following observations in support of this hypothesis. 1)

Morphine up-regulates production of IL-6 and TNF-α, and down-regulates IFN-γ production by human blood-derived monocytes; 2) Morphine, in the presence of SP, induces higher TNF-α production compared to morphine treatment alone; 3) Morphine and SP have a synergistic effect on expression of CCR-5 and CD4 in human promonocytic cells (U937). In addition, we observed that morphine enhances CXCR4 expression by human peripheral blood lymphocytes, which is related to the increased HIV replication in these cells treated with morphine. 4) Morphine in combination with SP augments HIV replication in human blood-derived macrophages, which can be blocked by either morphine or SP receptor antagonists (Fig. 1).

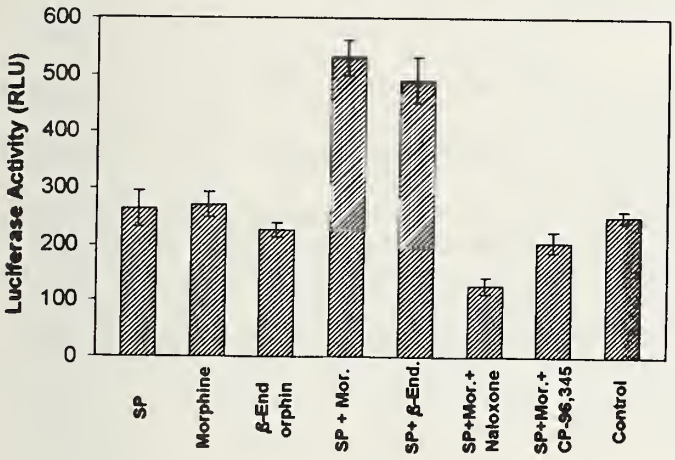

Fig. 1. Macrophages treated with SP (10^{-8} M) and/or morphine (10^{-10} M), or β-endorphin (10^{-10} M), or naloxone (10^{-8} M) or CP-96,345 (10^{-8} M) for 2 h before HIV ADA infection. Luciferase activity was quantitated in cell lysates 72 h after ADA infection. The data shown are means of triplicate cultures representative of 4 experiments.

5) Methadone, a drug for treating heroin addiction, induced HIV replication in latently infected human immune cells, which could be enhanced by SP. Taken together, the data described above indicate that morphine through its receptors on human immune cells up-regulates neuropeptide expression of SP and NK-1R, and that morphine and SP synergistically enhance HIV infection of human immune cells by altering the functions of these cells. These investigations into the complex relationship linking opioids with SP, and both substances with modulation of the host immune system and its defenses against HIV infection, will contribute to our basic understanding of host defense processes. Ultimately, these studies hold out the promise of furthering the development of improved treatments for opioid abusing patients infected with HIV or suffering from AIDS.

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SYMPOSIUM XI

NOVEL ANALGESIC AND NARCOTIC ANTAGONIST DRUGS

W. K. Schmidt[1] and F. Porreca[5], Chairpersons

Speakers: W. K. Schmidt,[1] R. L. Carpenter[2], A. T. McKnight[3], J. C. Lee[4], T. W. Vanderah[5], C. M. Flores[6]

[1]NorthStar Research & Development, Newark, DE; [2]Adolor Corporation, Malvern, PA; [3]Parke Davis, Cambridge, UK; [4]SmithKline Beecham, King of Prussia, PA; [5]University of Arizona HSC, Tucson, AZ; [6]University of Texas, San Antonio, TX

Recent developments in the understanding of pain and its neurobiological substrates have stimulated attempts to discover and develop new analgesics without the liabilities of abuse potential, GI disturbances and respiratory depression which restrict the chronic use of otherwise highly effective opioid agonists. This symposium was designed to afford a glimpse of new approaches that may augment or replace mu agonist therapy in the management of chronic pain and to highlight new compounds that may have applications to the treatment of opioid abuse.

Dr. William Schmidt (NorthStar) began the symposium with an overview of newly introduced analgesic drugs and the market dynamics that have shaped the introduction of new products during the past 5 years. He also reviewed drugs that are in advanced clinical development and others that have been discontinued by their sponsors during the past three years. Dr. Randall Carpenter (Adolor) profiled ADL 8-2698, a new peripherally-restricted mu antagonist that has demonstrated initial clinical activity in reversion opioid bowel dysfunction without antagonizing opioid analgesia. Dr. Sandy McKnight (Parke Davis) reviewed the ORL_1-nociceptin/orphanin FQ system as a target for novel analgesic drug development. Dr. John Lee (SmithKline Beecham) reviewed recent progress in developing peripherally-restricted cannabinoid CB2 ligands as analgesics. Dr. Todd Vanderah (Univ. Arizona) discussed recent work from his lab on new methods to modulate opioid tolerance development. And finally, Dr. Chris Flores (University of Texas) discussed neuronal nicotinic receptors as targets for developing novel analgesic drugs.

Each of the papers is summarized in the individual contributions that follow.

INTRODUCTION: WINNERS & LOSERS IN ANALGESIC DRUG DEVELOPMENT '98-'00

W. K. Schmidt

NorthStar Research & Development, Newark, DE

Recent studies estimate that approximately 106 million patients experience moderate to severe acute or chronic pain annually in the U.S. (Datamonitor, 1999). The most frequent diagnoses include cancer pain, postoperative pain, back pain. Moderate to severe pain in these cases is defined as pain that is significant enough to impair the ability of a person to carry on a productive life. Millions of additional patients suffer from migraine, arthritis pain, and other types of aches and pains that may vary in intensity from mild to moderate to severe. Pain in general, and chronic pain in particular, presents serious health and economic problems if it is not treated adequately.

The market for all types of analgesic products increased 60% in the past 5 years from $5.7 billion in 1994 to $9.1 billion in 1999 (IMS Health, 1994, 1999). Much of the market growth has come with the introduction of new products such as COX-2 inhibitors (celecoxib, rofecoxib), triptan antimigraine compounds (sumitriptan, zolmitriptan, naratriptan, rizatriptan), and a novel centrally-acting analgesic (tramadol). The COX-2 inhibitors, introduced in 1999, achieved total year-end U.S. sales of $1.5 billion which is equal to the market size for all NSAID analgesics combined. Additional growth came from new special use products to treat rheumatoid arthritis, osteoarthritis, and postherpetic neuralgia.

Substantial market growth has also come from newer formulations of existing products with more convenient or more effective dosage forms than previously available. Newer formulations of controlled-release narcotics achieved a 40% compounded annual growth rate between 1994 and 1999, indicating increased utilization of older products in

new dosage forms appropriate for chronic pain control. Five controlled release narcotic formulations achieved sales in excess of $1.1 billion in 1999.

The following tables summarize new analgesic product introductions from 1995 to the first half of the year 2000:

TABLE 1: New Narcotic-Related Analgesics U.S. Market (1995-2000)

Compound	Sponsor	Introduced
Tramadol, ULTRAM	Ortho-McNeil	1995
Oxycodone, OXYCONTIN	Purdue Pharma	1996
Morphine, KADIAN	Faulding / Zeneca	1996
Remifentanil, ULTIVA	Glaxo Wellcome	1996
Hydrocodone + ibuprofen VICOPROFEN	Knoll	1997
Fentanyl, ACTIQ	Anesta / Abbott	1999

TABLE 2: New Anti-Inflammatory Analgesics & Antiarthritic Drugs (1995-2000)

Compound	Sponsor	Introduced
Naproxen, NAPRELAN	Wyeth-Ayerst	1996
Bromfenac sodium, DURACT	Wyeth-Ayerst	1997
Leflunomide, ARAVA	Aventis	1998
Etanercept, ENBREL	Immunex / Wyeth	1998
Infliximab, REMICADE	Centocor	1999
Celecoxib, CELEBREX	Searle / Pfizer	1999
Rofecoxib, VIOXX	Merck	1999
Meloxicam, MOBIC	Boehringer Ingl.	2000

TABLE 3: New Anti-Migraine & Special Use Analgesics (1995-2000)

Compound	Sponsor	Introduced
Sumitriptan, IMITREX	Glaxo Wellcome	1995
Zolmitriptan, ZOMIG	Zeneca	1997
Naratriptan, AMERGE	Glaxo Wellcome	1998
Rizatriptan, MAXALT	Merck	1998
Hyaluronate, HYALGAN	Sanofi	1997
Hylan, SYNVISC	Sanofi	1997
Lidocaine patch, LIDODERM	Endo / Hind	1999

New products in advanced clinical development include the following compounds:

TABLE 4: Recent Advances in Anti-Inflammatory & Antiarthritic Drugs (1998-2000)

CLINICAL TRIALS (status):			
MK-633	COX-2 inhibitor	III	Merck
ML-3000	COX / 5-LO inhibitor	III	Merckle (Germany)
JTE-522	COX-2 inhibitor	II	Japan Tobacco / J&J
Anakinra	IL-1 receptor antagonist	III	Amgen
NCX-4016	NO-aspirin	II	NicOx

TABLE 5: Recent Advances in Narcotic-Related Analgesics (1998-2000)

CLINICAL TRIALS (status):

MorphiDex	Morphine + dextrometh.	NDA	Algos
Propiram	Partial μ agonist	III	Shire / Roberts
Fentanyl	E-TRANS delivery	III	Alza
ADL 10-0101	Peripheral κ agonist	II	Adolor
ADL 2-1294	Peripheral μ agonist	II	Adolor
Morphine 6-G	Morphine metabolite	II	CeNeS / ML Labs
DPI-3290	δ-μ agonist	I/II	Delta / Organon

Not all new products have achieved market success. Indeed, bromfenac (DURACT) was withdrawn from the market in 1998 due to severe liver failure, which was fatal in several instances where patients continued taking the drug beyond the label indication of 10 days or shorter periods of use. Some other products in clinical development have not achieved NDA approval (e.g. tenidap, a COX/5-LO inhibitor) or have been withdrawn from development following disappointing phase II clinical results or where the sponsor determined that they would not be competitive in the market compared to other new products.

In conclusion, the following factors have played a key role in promoting the development of new analgesic products:

- Large unmet medical need for safer, more effective analgesic drugs with fewer side effects
- Market responds robustly to introduction of new, clearly differentiated products
- Narcotics (DEA scheduled) and COX-1/COX-2 (unscheduled) analgesic drugs continue to have major roles in the treatment of acute & chronic pain
- Newer special use analgesics are playing increasingly important roles in the treatment of migraine, arthritis, and cancer pain
- Drugs with novel mechanisms of action will be increasingly important in the future

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Pharmaprojects, Scrip, and company reports

ADL 8-2698, A GUT-SELECTIVE MU ANTAGONIST DRUG FOR TREATMENT AND PREVENTION OF NARCOTIC-INDUCED CONSTIPATION AND G.I. SIDE EFFECTS; VALUE IN TREATING PAIN AND ADDICITON

R. L. Carpenter

Adolor Corporation, Malvern, PA

Opioids have profound and widespread effects on gastrointestinal function. In addition to the widely recognized inhibition of gastrointestinal motility, opioids also increase tone in smooth muscle within the bowel wall and sphincters, diminish secretions and increase fluid absorption. These gastrointestinal effects may prove beneficial for certain conditions such as diarrhea, but more frequently result in undesirable side effects.

Constipation is a universal side effect of opioid therapy. Tolerance to the constipating side effects of opioids develops slowly and remains incomplete for many patients (Vanegas *et al.*, 1998). Current therapy for opioid induced constipation (e.g. stool softeners, laxatives, enemas and/or bulking agents) has limited efficacy for most patients. In some patients, constipation becomes so severe as to limit the dose of opioid treatment.

Gastrointestinal Effects of Opioids: Opioid Bowel Dysfunction

	Pharmacologic Effects	Clinical Effects
Inhibition of Motility	Decreased gastric motility	Delayed gastric emptying Increased gastroesophageal reflux
	Inhibition of propulsive contractions in the small intestine	Delayed digestion of food Delayed absorption of oral medications
	Inhibition or abolition of propulsive contractions in the large intestine	Decreased frequency of BMs Straining with BMs Incomplete evacuation of stool Bloating and abdominal distention
	Increased bowel muscle tone and enhanced amplitude of non-propulsive segmental contractions	Spasm Abdominal cramps and pain
	Constriction of the sphincter of Oddi	Biliary colic, epigastric discomfort
	Increased tone of anal sphincter, reduced relaxation reflex with rectal distention	Impaired ability to evacuate the bowel
Altered Fluid Balance	Diminished gastric, biliary, pancreatic and intestinal secretions. Increased absorption of water from bowel contents	Hard, dry stool

Reference: Goodman and Gilman

One novel therapeutic approach for selectively antagonizing the GI effects of systemic opioids has been to orally administer opioid antagonists which have limited systemic absorption. For example, oral administration of the parenteral formulation of naloxone results in negligible plasma concentrations of naloxone (1-5% oral bioavailability). Clinical trials have clearly demonstrated that, with careful titration of the oral dose, it is possible to produce laxation without producing CNS opioid withdrawal in some patients (Culpepper-Morgan *et al.,* 1992). However, systemically absorbed naloxone freely enters the CNS. Consequently, some patients develop signs of CNS withdrawal or experience antagonism of analgesia at doses below those necessary to produce laxation (Sykes 1996; Latasch *et al.,* 1997).

An alternative strategy has been to attempt to selectively antagonize opioid effects on the GI tract. For example, opioid antagonists that are not absorbed systemically or do not cross the blood-brain barrier could selectively antagonize the adverse gastrointestinal effects. Preclinical studies clearly demonstrate the utility of this approach for selectively antagonizing the GI effects of systemic opioids (Russell *et al.,* 1982). However, clinical application of this principle has met with mixed results. For example, all patients in the initial clinical trials with nalmefene glucuronide developed opioid abstinence syndrome (Cheskin *et al.,* 1995). The abstinence syndrome was presumed to result when intestinal bacteria cleaved the glucuronic acid from the nalmefene glucuronide, and nalmefene was then systemically absorbed. In contrast, the only published trial of methylnaltrexone in patients to date has demonstrated excellent efficacy (Yuan *et al.,* 2000). Intravenous administration of methylnaltrexone produced laxation in all eleven subjects with severe opioid bowel dysfunction due to chronic methadone maintenance pharmacotherapy.

ADL 8-2698 is a novel competitive opioid antagonist which combines the above strategies to selectively antagonize adverse GI effects of systemic opioid pharmacotherapy. Systemic absorption of orally administered ADL 8-2698 is one to two orders of magnitude lower than for naloxone (0.05% in the dog). It also has limited ability to cross the blood-brain barrier. When administered intravenously to opioid tolerant rats, the dose required to antagonize opioid CNS effects is >100 times higher than the dose required to antagonize the GI effects (Zimmerman *et al.,* 1994). Clinical trials with orally administered ADL 8-2698 demonstrate clear efficacy in patients experiencing severe constipation associated with chronic opioid therapy for the treatment of pain or addiction. ADL 8-2698 reliably produced laxation in these patients and a clear dose response is apparent. Patients did not develop signs of CNS withdrawal or a reduction in analgesia. ADL 8-2698 shows promise for treatment of adverse gastrointestinal side effects of opioid therapy.

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THE ORL_1 RECEPTOR: A NEW TARGET FOR ANALGESIC DRUG DEVELOPMENT?

A .T. McKnight, S. L. Mason, J. R. Nicholson and M. Ho

Pfizer Global Research & Development, Cambridge Laboratories, Cambridge University Forvie Site, Robinson Way, Cambridge, UK

Introduction

The existence of distinct μ-, κ-, and δ-opioid receptors was well established before molecular cloning demonstrated the structural basis for the pharmacological homology between the different types. The amino-acid sequences were about 60% identical overall, and more than 70% identical in the membrane-spanning regions. Extending the screening of genomic and cDNA libraries led to the identification of a novel receptor with as high a degree of homology towards the "classical" opioid receptor types, as they shared among each other. Although the putative receptor has had as many names as the groups reporting its identification (see Henderson and McKnight 1997), there is consensus for the use of the original designation for the human form: ORL_1. This receptor was accepted into the opioid receptor family on the basis of its structural homology, however there is no corresponding pharmacological homology; even non-selective ligands with uniformly high affinity towards μ-, κ- and δ-receptors have generally very low affinity for the ORL_1 receptor.

The discovery of the endogenous agonist was made simultaneously by two groups (Meunier *et al.* 1995; Reinscheid *et al.* 1995), in what is held to be one of the "first examples of the successful application of reverse pharmacology or functional genomics" (Meunier 2000). Both labs reported the first observation of the (presumed) pharmacological consequence of activation of the ORL_1 receptor in the brain: reduced response latency to a noxious-thermal stimulus. This pro-nociceptive effect was the rationale for naming the endogenous agonist "nociceptin" by one group (Meunier *et al.* 1995), but the field is divided in its preferred terminology, with "orphanin FQ" (Reinscheid *et al.* 1995) being used with roughly equal frequency. Nociceptin/orphanin FQ (hereafter N/OFQ) is a 17-amino acid peptide with homology to known endogenous opioid peptides, particularly dynorphin A. The greatest sequence homology is at the N-terminus, with the Gly^2-Gly^3-Phe^4 motif common to all the opioid peptides in mammals, but the most striking feature is the dissimilarity. N/OFQ has Phe as its N-terminal amino acid, where the known opioid peptides have Tyr, which explains the low affinity of N/OFQ at μ-, κ- or δ receptors.

The pharmacology of the ORL_1-nociceptin/orphanin FQ system

N/OFQ and the ORL_1 receptor are widely distributed in the CNS (see Henderson and McKnight 1997; Meunier 2000) and consequently a role has been proposed for the N/OFQ-ORL_1 system in the control of many neuro-behavioural functions. Firm pharmacological evidence is lacking however for the want of good experimental tools, or is contradictory. Nowhere is this more apparent than in the context of pain, where pro-nociceptive and anti-nociceptive effects are described at both spinal and supraspinal sites (see Meunier 2000).

The original report of hyperalgesia after icv administration of N/OFQ has been attributed to block of stress-induced analgesia mediated by release of endogenous opioids (Mogil *et al* 1996a); the "anti-opioid" action of icv N/OFQ extends to exogenous opioids (Mogil *et al.* 1996a,b). N/OFQ may have a role in the integration of adaptive responses to stressful stimuli (Jenck *et al.,* 2000), so a clear view of the effect of N/OFQ in pain control may be obtained only if confounding environmental influences are absent, or are carefully controlled (Darland *et al.* 1998).

It must also be considered that in some cases (perhaps depending on timing, dose or site of injection) the effects following administration of N/OFQ may not involve activation of the ORL_1 receptor. The peptide is susceptible to breakdown, after icv injection at least (see Figure 1), and fragments that are inactive at the ORL_1 receptor may have effects of their own, or may interfere with the action of N/OFQ. Recently the N-terminal fragment nociceptin(1-7) was reported to block the hyperalgesia produced by intrathecal injection of N/OFQ in the rat (Sakurada *et al.* 1999). It will be important to repeat the original work with N/OFQ using non-peptide ORL_1-receptor agonists such as Ro-64-6198 (Figure 3), when these become available. To date only the anxiolytic properties of Ro-64-6198 have been illustrated, although the compound is reported to have no effect on tactile sensitivity in the rat, or on response latency in the tail-flick test (Jenck *et al.*, 2000).

Another complicating factor with the use of N/OFQ given icv is that motor dysfunction is invariably encountered (Figure 1). Consideration should be given to this, since end-point measurement in pain tests generally relies on motor performance. This action seems to be involve the ORL_1 receptor since we see a similar effect with the N/OFQ analogue [$Phe^1\psi(CH_2\text{-}NH)Gly^2$]nociceptin(1-13)$NH_2$, or with the more potent partial agonist acetyl-Arg-Tyr-Tyr-Arg-Trp-Lys-NH_2, so it will also occur with a CNS-penetrating non-peptide agonist given systemically.

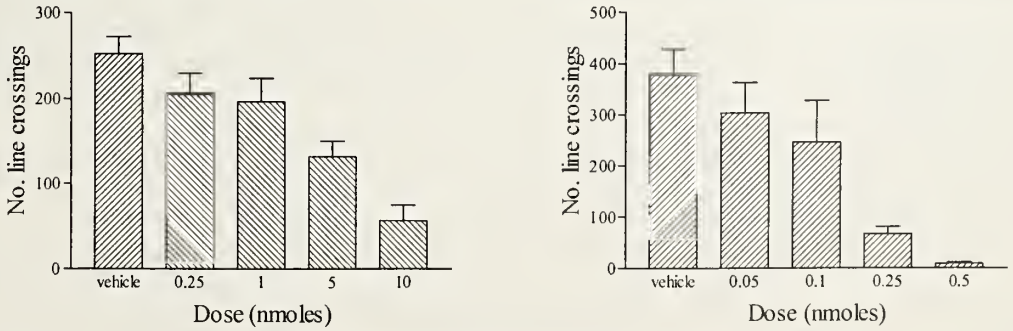


Figure 1 Depression of motor function by N/OFQ given icv in the rat
Line crossings for 60 minutes after administration of N/OFQ were profoundly depressed at 5-10nmols in aCSF vehicle (left), but with sub-nanomole doses when peptidase inhibitors were present (right).

Antagonists for the ORL_1 receptor

It is axiomatic that if a particular action or effect is attributable to activation of the ORL_1 receptor, there should be an observable block by a selective antagonist. So far, useful antagonists for the ORL_1 receptor have not been available. The synthetic analogue of N/OFQ [$Phe^1\psi(CH_2\text{-}NH)Gly^2$]nociceptin(1-13)$NH_2$ was first reported to be a selective antagonist (Guerrini *et al.*, 1998), but increased use of this peptide pointed to it having agonist actions (see Meunier, 2000), as considerations of its structure would support. Simply put, the ligand is a partial agonist at the ORL_1 receptor (Figure 2), although generally in peripheral tissues the efficacy is very low (Guerrini *et al.* 1998; Ho *et al.,* 2000). More recently, the related peptide [$Nphe^1$]nociceptin(1-13)NH_2 has been shown to lack efficacy (Hashimoto *et al.,* 2000), but affinity is low.

A peptide related to the hexapeptide acetyl-Arg-Tyr-Tyr-Arg-Trp-Lys-NH_2, but with isoleucine substituting for tryptophan, was reported to be an ORL_1 antagonist *in vitro* (Berger *et al.*, 1999), although the ligand was originally reported to be a potent partial agonist (Dooley *et al.*, 1997). Again the partial agonist nature of this ligand (Figure 2) will explain the appearance of antagonist activity, with lower or higher efficacy being obtained depending on the experimental conditions. Although the affinity in this hexapeptide class of ORL_1 ligands is high, they are highly susceptible to enzymatic degradation and cannot easily be employed for whole-animal studies.

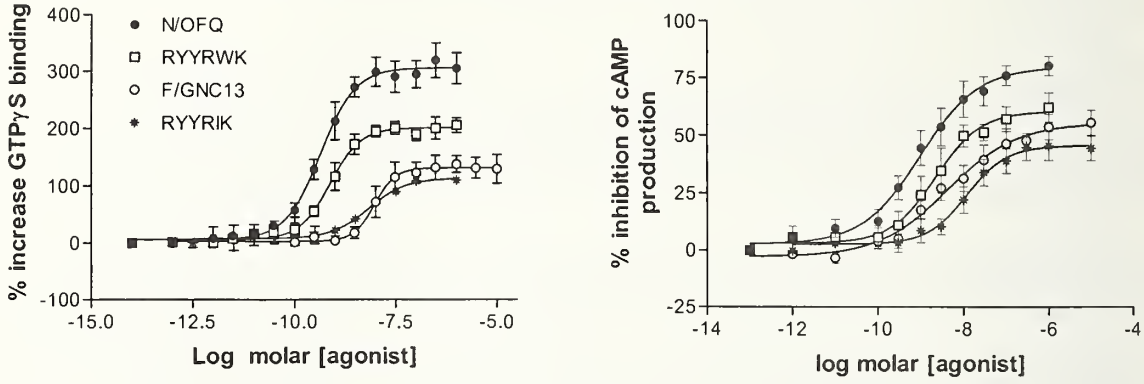
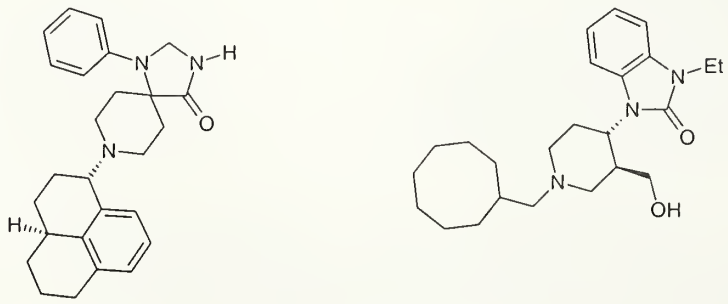


Figure 2 Potency and efficacy of peptide agonists at the rat ORL_1 receptor
The relative efficacies of the partial agonists acetyl-Arg-Tyr-Tyr-Arg-Trp-Lys-NH_2 (RYYRWK) and its Ile^5 analogue (RYYRIK), and [$Phe^1\psi(CH_2\text{-}NH)Gly^2$]nociceptin(1-13)$NH_2$ (F/GNC13) increase compared to the full agonist N/OFQ going from the GTPγS assay (left) to the cyclase assay (right), in keeping with classical receptor theory.

Reports of non-peptide antagonists for the ORL_1 receptor have begun to appear in the patent literature, and the activity *in vitro* of one example from the benzimidazolone series from Banyu has been reported. J-113397 (Figure 3) is reported to be a neutral antagonist at the human ORL_1 receptor expressed in CHO cells, with good affinity and 1000-fold selectivity for ORL_1 over the μ-receptor (Kawamoto *et al.*, 1999). The compound is reported to block a hyperalgesic effect of N/OFQ, but details of any activity of the compound by itself have not been given. When this or other such antagonists become available, along with non-peptide agonists of similar affinity and selectivity such as the triaza-spirodecanone Ro-64-6198 from Roche, the validity of the ORL_1 receptor as a target for analgesic drug discovery can be tested.



Ro 64-6198 **J-113397**

Figure 3 Non-peptide agonist and antagonist ligands for the ORL_1 receptor

Conclusions

The N/OFQ–ORL_1-receptor system has been implicated in the control or modulation of pain, although the pharmacological evidence is contradictory. This evidence comes largely from experiments in pain models involving the administration of the peptide to the brain or spinal cord, but the use of the exogenous peptide in this way is problematic. The peptide will be prone to enzymatic degradation, and effects unrelated to activation of the ORL_1 receptor are possible; this issue will be obviated in future when selective non-peptide agonists are available. Supraspinally N/OFQ has clear effects to depress motor function at doses that overlap with those used to explore other central actions, and this cannot fail to affect end-point measurement in pain tests. Such an action would also be a complication with the use of a CNS-penetrating non-peptide agonist given systemically, since it seems to be attributable to ORL_1 receptor activation. Potentially the most useful tools for the definitive proof-of-concept studies are selective non-peptide agonists, and these are now being described. The future availability of these ligands to the scientific community will enable the concept of the ORL_1 receptor as a target for the development of novel analgesic drugs to be tested.

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RECENT ADVANCES IN PERIPHERAL CANNABINOID CB2 AGONISTS AND ANTAGONISTS

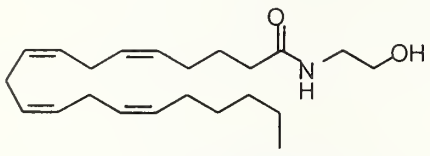
J. C. Lee

SmithKline Beecham Pharmaceuticals, 709 Swedeland Road, King of Prussia, PA

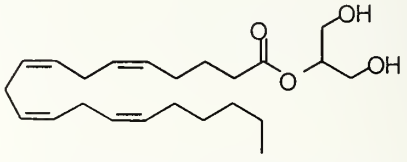
Cannabinoids have been suggested to mediate peripheral effects apart from their well-known CNS effects (Pertwee 1999). Until recently, our undcrstanding of cannabinoid pharmacology at the molecular and cellular level has been limited by a lack of receptor subtype selective tools. The first demonstration of the existence of a cannabinoid receptor by radioreceptor binding assay (Devane *et al.,* 1988) was quickly followed by the cloning and characterization of the central receptor (CB1) (Matsuda 1997) and the peripheral receptor (CB2) (Munro *et al.,* 1993).

Cannabinoid Ligands and Receptors

The two known cannabinoid receptor (CB) subtypes, CB1 and CB2, belong to the G-protein coupled receptor class and share 44 and 68 % sequence identity in their overall structure and putative transmembrane domains respectively. Both CB1 and CB2 bind cannabimimetic agonists with similar rank order potencies, HU210>CP 55940> WIN 55212 $\geq \Delta^9$THC>cannbinol>anandamide (Hanus *et al.,* 1999). The indole compound, WIN 55212-2 shows 19 fold selectivity for CB2, thus representing the first CB2 selective agonist. More recently, 2-Arachidonyl glycerol, an endocannabinoid, has been identified as a full CB2 agonist in stimulating GTPγS exchange that is attenuated by anandamide (Gonsiorek *et al.*, 2000).



Anandamide



***sn*-2-arachhidonyl glycerol**

Distribution of CB receptor mRNA

The predominant expression pattern of the two receptor subtypes suggests that peripheral and CNS effects of cannabinoids may be differentially mediated although there are tissues where both receptors are detected (Galiegue *et al.,* 1995). The distribution of CB receptor mRNA has been examined by Northern Blot analysis and RT-PCR. The CB1 subtype is expressed primarily in the CNS and testes (Matsuda *et al.,* 1993; Gerard *et al.,* 1991) whereas the CB2 subtype is expressed mainly in B lymphocyte-enriched areas such as the marginal zone of the spleen, with detectable levels in macrophages (Munro *et al.,* 1993), rat mast cells (Facci *et al.*, 1995) and human B cells (Derocq *et al.,* 1995).

Functional assays of recombinant receptor

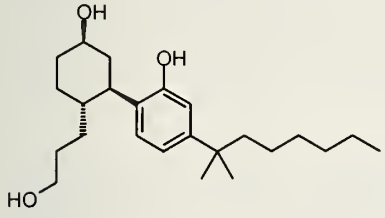
CB1 and CB2 receptors are negatively coupled to adenylyl cyclase through a pertussis toxin sensitive G protein (Slipetz *et al.,* 1995; Felder *et al.,* 1998). Different biochemical assays have been used to assess receptor activation including cAMP production, guanine nucleotide exchange, activation of MAP kinase and reporter techniques such as melanophore pigment aggregation (Nuttall *et al.,* 1999) and activation of luciferase transcription.

Alterations of CB receptor expression in disease model systems

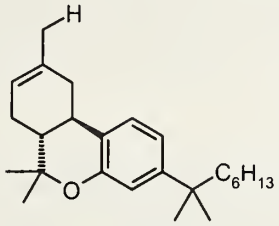
Few studies to date have attempted to relate the expression of the cannabinoid receptors or specific endogenous ligands to disease pathogenesis. Behavioral responses such as movement control have been suggested to be a result of decreased CB1 receptor expression in Huntington's disease (Richfield and Herkenham, 1994). In the rat renal failure model, a time-dependent increase in CB2 receptor and a concomitant decrease in CB1 receptor expression were observed in ischemic kidney (unpublished observations). More recently, genes regulated by CB2 receptor activation identified by microarray gridding in a promyelocytic cell line suggest its involvement in hematopoietic cell differentiation (Derocq *et al.,* 2000).

CB2 receptor agonist

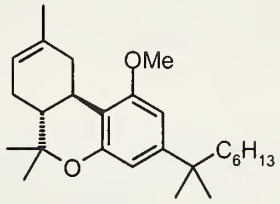
Through extensive SAR studies, it was demonstrated that the pharmacophore represented by CP 55,940 can be readily modified by replacing the phenolic hydroxyl with either a hydrogen or a methoxyl to increase potency and selectivity against the CB2 receptor. Examples include HU210 (Ki=0.032 nM with 10 fold selectivity) and L-579,633 and L-759,656 (Ki=20 nM with 1000 fold selectivity for both) (Xiang and Lee 2000).



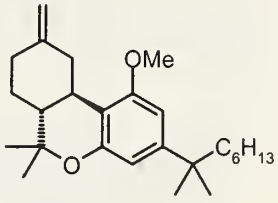
CP 55,940



HU210

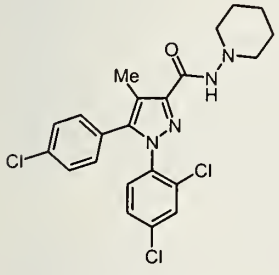


L-759,633

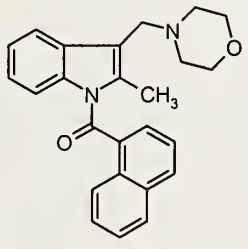


L-759,656

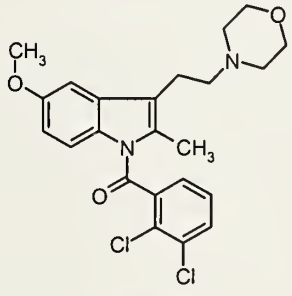
The design of new aminoalylindoles, of which WIN 551222-2 is the prototype (Ki=0.3 nM; 5 fold selective against CB2) has also progressed quite nicely yielding examples such as L-759,787 and L-768,242 with good Ki and much improved selectivity (Ki 10 nM and 100 fold selective for CB2)



WIN 551222-2



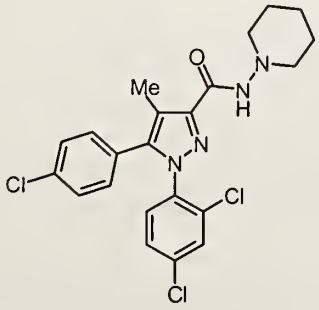
L-759,787



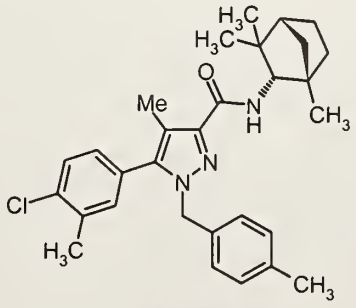
L-768,242

CB2 receptor antagonist

The first CB2 selective antagonist, SR 144526 was derived from a CB1 selective antagonist, SR 141716, of the diarypryazole class (Slipetz *et al.,* 1995; Rinaldi-Carmona *et al.,* 1995). By placing a benzyl group and a fenchylamide substituent at the 1 and 3 positions respectively of the pyrazole ring, the potency and selectivity profile is reversed. SR 144526 is a subnanomolar (Ki = 0.6 nM) and selective inhibitor (over 700 fold). The compound is orally active and is a functional antagonist in cells expressing the recombinant receptor as measured by inhibition of cAMP accumulation and MAP kinase activation. *In vitro*, the compound also inhibited ligand induced B cell proliferation. Interestingly, this and other CB2 receptor antagonists also act as inverse agonists as the compound alone inhibits basal incorporation of GTP-γS (Bouaboula *et al.,* 1999a), MAP kinase activation (Bouaboula *et al.,* 1997), and receptor phosphorylation (Bouaboula *et al.,* 1999b).



SR 141716



SR 144528

Conclusions

Using the available receptor selective agonists and antagonists, it is possible to associate some of the inflammatory/immunoregulatory effects to with the CB2 receptor. However, attempts to demonstrate unequivocally the functional utility of these potent and selective compounds have been rather disappointing. Furthermore, the "inverse agonist" effect seen with the antagonists to both receptors may limit their clinical utility. It is hoped that additional pharmacological studies using improved tool compounds or genetic approaches (e.g. gene deletion) will help to better define the role of CB2 receptors in health and disease.

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DESCENDING FACILITATION IN OPIOID-INDUCED PAIN AND ANTINOCICEPTIVE TOLERANCE

T. W. Vanderah, M. H. Ossipov, T. P. Malan, Jr.; J. Lai and F. Porreca

Departments of Pharmacology and Anesthesiology, University of Arizona HSC, Tucson, AZ

States of abnormal pain induced by injuries to peripheral nerves and opioid tolerance share common features including tactile allodynia and hyperalgesia of the hindpaws, decreased spinal opioid antinociception and loss of supraspinal/spinal opioid antinociceptive synergy. Modulation of pain by descending inhibitory and faciliatory systems from the rostral ventromedial medulla (RVM) are well known leading us to study possible RVM modulation of opioid-induced pain and tolerance. In s.c. morphine (MS)-, but not placebo-, implanted rats, tactile allodynia and hyperalgesia were observed; this abnormal pain was blocked by RVM injection of lidocaine suggesting tonically active descending facilitation. The i.th. MS antinociceptive dose-response curve (DRC, tail-flick) was displaced to the right in MS-pelleted rats, indicative of tolerance; this right shift in the DRC was blocked by RVM lidocaine in a time-related manner. Similarly, the s.c. MS antinociceptive DRC was displaced to the right in MS pelleted rats and this right shift was also blocked by RVM lidocaine, suggesting restoration of supraspinal/spinal antinociceptive synergy. As animals implanted with MS pellets displayed hyperalgesia, the experiments were repeated by normalizing the foot-flick threshold to control levels by reducing the stimulus intensity. At predrug baseline responses elicited by lower stimulus intensity, the i.th. morphine DRC (foot-flick) in MS pelleted rats was not different from placebo. The data suggest that chronic exposure to opioids results in increased pain which requires a higher opioid dose at the spinal level accompanied by a loss of supraspinal/spinal opioid synergy. Increased pain and opioid tolerance appear to result from tonic activity of descending facilitation from the RVM offering approaches to limit opioid "tolerance."

NEURONAL NICOTINIC RECEPTORS AS TARGETS FOR ANALGESIC DRUG DEVELOPMENT

C. M. Flores

Departments of Endodontics and Pharmacology, The University of Texas Health Science Center at San Antonio, San Antonio, TX

Although nicotine's antinociceptive properties have been appreciated for the better part of a century (Davis *et al.*, 1932), its poor therapeutic index has precluded its clinical development as an analgesic. More recently, however, discoveries of both naturally occurring (epibatidine) and synthetic (ABT-594) antinociceptive agents with extremely potent, broad-spectrum and, perhaps, safer profiles have rejuvenated considerable interest in exploiting a nicotinic cholinergic approach to pain control (Spande *et al.*, 1992; Bannon *et al.*, 1998). Such efforts are being further enhanced by an expanding appreciation of the heterogeneity that exists within the family of nicotinic acetylcholine receptor subtypes and the functions they subserve. Nonetheless, significant obstacles remain before this novel pharmacological approach to pain management will reach fruition. Principally, these include toxicity, duration of action and variability in analgesic response. To overcome these obstacles, it will be critically important to identify, on both cellular/molecular and systemic levels, those determinants which underlie them. Such efforts will be greatly facilitated by a comprehensive description of the repertoire of nicotinic receptor subtypes, the extent to which these may exhibit plasticity (e.g. under conditions of injury or chronic drug treatment), their anatomical localization in the central and peripheral nervous systems and their biophysical properties, especially with respect to desensitization

and inactivation processes. To accomplish these goals, more selective and powerful tools to probe these macromolecules are required. Collectively, these advances will lead to a more thorough elucidation of the spectrum of antinociceptive action by nicotinic agents as well as the mechanisms and pathways they engage.

Myriad investigations by several laboratories have documented the antinociceptive efficacy of a variety of nicotinic cholinergic agonists in several species, including man (for review, see Flores and Hargreaves, 1998). Many of these studies, primarily using microinjection approaches in conjunction with pharmacological antagonists, have systematically elucidated many of the anatomical pathways and neurotransmitter systems utilized to produce antinociception via neuronal nicotinic receptor activation. Nominally, these appear to involve, *inter alia*, stimulation of serotonergic, adrenergic as well as cholinergic descending, pain inhibitory fibers. Unfortunately, as alluded to above, there are relatively few subtype-specific agents available that would allow for the precise determination of the nicotinic receptor populations involved. Nonetheless, antisense (Bitner *et al.,* 2000) and knock-out (Marubio *et al.,* 1999) strategies have been used successfully to implicate a prominent contribution of the $\alpha4\beta2$ nicotinic receptor subtype that has been shown to predominate in the CNS (Flores *et al.,* 1992). Further investigations in our laboratory have directly described an additional subtype, $\alpha3\beta4$, which appears to constitute a major proportion of nicotinic receptors found in sensory neurons (Flores *et al.,* 1996), thereby providing a target for a potential, peripherally acting nicotinic analgesic (see below).

A compelling attribute of nicotinic antinociception is the spectrum of nociceptive states across which these agents exhibit efficacy. Nominally, these include both inflammatory and neuropathic pain models involving the three major modalities of noxious thermal, mechanical and chemical stimulation. It is worth noting that, among humans, painful conditions involving the head and face affect up to a quarter of the U.S. population (Lipton *et al.,* 1993). It will be important, therefore, to identify new analgesics that show efficacy in the treatment of pain within the trigeminal representation. Recently, we demonstrated in the orofacial adaptation of the formalin model that epibatidine exhibited dose-dependent, nicotinic receptor-mediated antinociception in both the acute and tonic phases of the formalin test (Gilbert *et al.,* in press). Thus, nicotinic agonists show activity in animal pain models involving trigeminal as well as spinal innervation sites, presaging an even wider spectrum of analgesic activity in humans.

Among all of the potential impediments to the realization of a nicotinic therapeutic, perhaps none garners so much attention as potential toxicity. And although nicotine, for example, can produce motoric effects, convulsions and even death at high enough doses, in particular, there is a concern for abuse potential. To the extent that the phenomena associated with dependence and substance abuse are known to be mediated primarily within the CNS, the potential for developing a peripherally active nicotinic analgesic is immediately appealing. Indeed, it was shown that nicotine-induced antinociception in the tail withdrawal assay was blocked by the peripherally active nicotinic receptor antagonist chlorisondamine (Caggiula *et al.,* 1995). In support of a possible peripheral site of action for a would-be nicotinic analgesic, we have used the *in vitro* superfusion of peripheral nerve terminals to evaluate the modulation of nociceptor function by a variety of pharmacologically distinct agents that act at nicotinic receptors. Thus, we have shown that ABT-594 is capable of inhibiting capsaicin-evoked calcitonin gene-related peptide (CGRP) from peripheral nociceptor terminals in rat buccal mucosa by up to 60% (Dussor *et al.,* 1998). And this is consistent with original reports that ABT-594 inhibits capsaicin-evoked substance P release from central nociceptor terminals in spinal cord slices *in vitro* and, when administered peripherally to anesthetized animals, inhibits electrophysiological responses of dorsal horn neurons following noxious stimulation (Bannon *et al.,* 1998). As mentioned above, sensory ganglia express nicotinic receptors, including $\alpha3\beta4$ and $\alpha4\beta2$ subtypes, and their neuronal distribution includes nociceptors, thereby providing a molecular basis for direct regulation by nicotinic agonists (Flores *et al.,* 1996). In addition, sensory neurons express alpha-bungarotoxin binding (probably $\alpha7$) nicotinic receptors (Polz-Tejera *et al.,* 1983) further increasing the heterogeneity of potential targets in the periphery. Taken together, these data establish the rationale and potential viability of a peripherally selective nicotinic analgesic agent which, if efficacious, would predictably exhibit a much improved safety profile, including a greatly diminished abuse liability. In addition, there is evidence that nicotine exhibits synergistic actions with morphine (Suh *et al.,* 1996) giving rise to the possibility of using a nicotinic analgesic as an opioid sparing adjunct that would seek to mitigate the untoward effects of both drugs.

Finally, an emerging theme in experimental and clinical pharmacology is that of pharmacogenetics, which refers to genetic-based variability in drug response, particularly with regard to analgesia (for review, see Mogil, 1999). This variability derives from one or more genes that are involved in the pharmacokinetic disposition and or pharmacodynamic action of a given drug. In fact, one of the better known examples of this phenomenon comes from the field of pain management in which up to 10% of patients who are "poor metabolizers" experience little or

no relief from codeine because of a polymorphism in the CYP2D6 gene that is responsible for converting this prodrug into morphine (Persson *et al.,* 1995). Following up on this concept, we evaluated whether the nicotinic agonist epibatidine exhibits pharmacogenetic variability with respect to its antinociceptive effects. In fact, among 8 inbred mouse strains, we observed significant differences in both the magnitude and duration of epibatidine-induced antinociception in the tail withdrawal assay, with calculated potency differences between strains as high as 20-fold (Flores *et al.,* 1999). Of particular interest was the demonstration that, in contrast to the relatively short duration of antinociception (approximately 15 minutes) observed among all other strains examined, the A/J strain exhibited significant antinociception for at least three hours following epibatidine administration. This has important implications for the pharmaceutical development of this drug class insofar as the vast majority of studies on nicotinic receptor-mediated antinociception have similarly described a remarkably short duration of drug action, most probably reflecting tachyphylaxis due to receptor desensitization. Thus, a better understanding of the genes that determine duration of antinociceptive action could lead to the development of a longer acting analgesic.

In addition, there is accumulating data to suggest that there may be gender-based differences in nicotinic antinociception. For example, Craft and Milholland (1998) found in the rat hotplate test that nicotine, at every dose tested, produced greater antinociception in females than in males. Similarly, it was shown that intrathecally administered metanicotine (RJR 2403) or neostigmine was significantly more potent in producing antinociception in females compared with males and that in the former but not the latter, the effects of neostigmine were partially antagonized by mecamylamine (Chiari *et al.,* 1999). In contrast, however, Jamner *et al.* (1998) found that among humans, nicotine produced analgesia to electrocutaneous stimulation in male but not female smokers and non-smokers. Thus, if indeed we do progress to the point of one or more nicotinic analgesics entering the clinical armamentarium, then we ought to be prepared for the probability that patients will experience subtle to significant differences in pharmacological effect due to genetic factors including gender. Obviously, this would be one case for which the advent of DNA screening could have direct, beneficial impact on patient treatment.

In summary, the potential for a nicotinic cholinergic class of analgesic drugs appears increasingly viable. Indeed, although there are substantial hurdles to realizing this potential, ranging from toxicity and abuse potential to duration of action and pharmacogenetic variability, the evidence to date suggests that these challenges may be overcome. The wide spectrum of action exhibited by nicotinic agonists across pain models is virtually unprecedented, holding out the promise for improved efficacy over existing therapies against the most intractable pains. Moreover, the demonstration of synergistic actions provides an opportunity for toxicity-limiting, adjunctive therapeutic strategies. Most importantly, these collective investigations constitute the legitimate prospect for realizing better and safer pain management via a novel approach rooted in nicotinic cholinergic pharmacology.

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SYMPOSIUM XII

INTERACTION OF DRUGS OF ABUSE AND CHEMOKINE RECEPTORS IN RELATIONSHIP TO AIDS

T. J. Rogers and R. M. Donahoe, Chairpersons

A growing body of literature has established that the common drugs of abuse exert a profound influence on the immune response. However, the cellular and molecular basis for these effects remain largely uncertain at this time. Because of the relationship between drug abuse and infection with HIV, a number of investigators have begun to examine the role of opioid and cocaine administration on the immune response to this critically important virus. The studies reviewed here describe the capacity of these drugs to alter several critical components of the host-virus interaction, including the expression of HIV coreceptors, inflammatory cytokine expression and cellular migration. It is clear that we have illuminated only a small part of the influence of drugs of abuse on the immune response in general, or of the interaction of HIV with the immune system specifically.

DUAL REGULATION OF CHEMOKINE AND OPIOID RECEPTORS, IMPACT OF HIV INFECTION

T. J. Rogers, M. A. Wetzel and E. E. Henderson

Temple University School of Medicine, Philadelphia, PA

INTRODUCTION

Based on prior studies showing the capacity of μ-opioids to alter pro-inflammatory cytokine expression, we hypothesized that opioid administration might alter chemokine production. Our data show that the μ-selective agonist [D-ala^2, *N*-Me-Phe^4, Gly-ol^5]enkephalin (DAMGO) elevates both the mRNA transcripts and protein expression of the CC-chemokines, monocyte chemoattractant protein (MCP)-1, and RANTES, as well as the CXC-chemokine IFN-γ-inducible protein (IP)-10, in PBMC cultures. Since MCP-1, RANTES, and IP-10 are potent chemoattractants for both monocytes and certain populations of lymphocytes, this upregulation of chemokine expression by opioids may influence trafficking of potential non-infected, target cells to the site of active infection. In this way, we speculate that enhanced expression of MCP-1, RANTES, and IP-10 may directly contribute to HIV-1-induced T cell depletion leading to immunosuppression, pathogenesis and progression to AIDS.

RESULTS AND DISCUSSION

In an effort to better understand the influence of μ-opioids on cytokine production, we investigated the effect of DAMGO administration on the expression of chemokines by human PBMCs. The results showed that the DAMGO treatment induced the production of MCP-1, IP-10 and RANTES. In an effort to characterize the mechanism of DAMGO-induced upregulation of chemokine protein levels by PBMCs, we carried out experiments to quantitate chemokine mRNA levels after DAMGO treatment. RNAse protection analysis showed that DAMGO administration induced a significant increase in the levels of RANTES, IP-10 and MCP-I mRNA, suggesting that the opioid acts at the level of transcription. In addition, we were interested in whether DAMGO induction of chemokine expression was mediated through the μ-opioid receptor, and pretreatment with the μ-opioid-selective antagonist CTAP was found to abolish the DAMGO-induced increase in chemokine expression.

Evidence suggests that chemokines differentially regulate HIV replication during HIV disease progression to AIDS. Those chemokines that are ligands for the CCR5 and CXCR4 receptors have been shown to inhibit viral replication by competing with HIV for binding to the HIV coreceptors (Peterson *et al.,* 1987). However, it is important to note that recent studies have also shown that pretreatment of T cells with β-chemokines MIP-1α, MIP-1β, and RANTES increases the absorption and replication of some T-tropic HIV strains (Dolei *et al.,* 1998). In view of these findings, and the established capacity of μ-opioids to augment the replication of HIV-1 in vitro (Peterson *et al.,* 1990), we tested the effect of DAMGO on chemokine production by HIV-1-infected PBMCs. The results showed that both M- and T-tropic HIV infection alone augmented the levels of MCP-1. However, DAMGO pretreatment of T-tropic HIV-1-infected cells resulted in a significant increase in RANTES and IP-10, but not MCP-1, levels. Treatment with

as little as 0.1 nM DAMGO induced a 7-fold increase in IP-10 protein levels in T-tropic HIV-infected PBMCs. DAMGO treatment exerted differential effects on RANTES and IP-10 protein expression. Specifically, in M-tropic HIV-infected PBMCs, administration of DAMGO at a high concentration (1 μM) significantly elevated RANTES expression by at least 13-fold, while levels as low as 1 nM reduced IP-10 expression 3-fold. Our results suggest that DAMGO administration differentially regulates RANTES and IP-10 protein expression in HIV-infected PBMCs, and this appears to be dependent on viral tropism.

These findings provide additional evidence that μ-opioids modulate immune function by altering the production of pro-inflammatory chemokines by cells of the immune system. Our results are consistent with earlier studies from several laboratories that have shown that endogenous endorphins and enkephalins increase the production of pro-inflammatory cytokines, including IL-1, IL-2, and IFN-γ (Brown and Van Epps, 1986). These findings are complicated by the fact that these opioids are not highly selective for a particular opioid receptor class. Moreover, our results contrast with the results of studies on the effect of morphine on cytokine expression. For example, studies reported by Peterson *et al.,* (1987) show that IL-2 and IFN-γ production is inhibited following morphine administration. Chao *et al.* (1992) demonstrated a significant increase in TGFβ production following morphine treatment of LPS- or PHA-activated PBMCs. It is well documented that TGFβ elicits many opposing cellular effects, depending on the cell type, and maturation-differentiation status of the responding cell. The documented immunosuppressive activity of TGFβ may explain the inhibition of IL-2 and IFN-γ production following morphine administration. Our data support the notion that μ-selective opioids are immunomodulatory and have the capacity to enhance the production of MCP-1, IP-10, and RANTES by cells of the immune system.

The correlation between drug abuse in general, and heroin abuse specifically, with HIV-1 infection is well established. Morphine is a major breakdown product of heroin, exhibits μ-opioid agonist activity, but is not a selective μ-opioid agonist. Studies by Peterson *et al.,* (1990), showed morphine administration potentiated HIV-1 replication in human PBMCs. These findings suggested that opioids could act as cofactors in the pathogenesis of HIV-1 in intravenous drug users. However, the mechanism of immunomodulation by opioids during HIV-1 infection is still not fully understood. Indeed, conflicting results have been reported for the effect of morphine on the progression of SIV infection in monkeys (Chuang *et al.,* 1993a; Donahoe. *et al.,* 1993). The disagreement in the results from these primate studies could be due to differences in the SIV strain and in morphine dosages employed by these laboratories. Our data suggest that opioids may enhance HIV-1 replication through modulation of chemokine expression. It appears that at high concentration, CC-chemokines RANTES, MIP-1α, and MIP-1β can act as HIV-1-suppressive molecules in $CD4^+$ T cells by competitively binding HIV-1 coreceptors required for entry into target cells (Cocchi *et al.,* 1995). In contrast, several studies show that chemokines may exhibit enhancing rather than inhibitory effects on HIV-1 replication (Dolei *et al.,* 1998; Kelly *et al.,* 1998). For example, Dolei *et al.* (1998) determined that pretreatment of T cells with RANTES, MIP-1α, and MIP-1β increased the replication of T-tropic HIV-1 strains in a dose-dependent manner. Gordon *et al.,* (1999) demonstrated enhancement of both M- and T-tropic HIV-1 infection when RANTES was administered prior to or simultaneously with HIV-1 infection. The mechanism responsible for enhanced viral replication by RANTES may be related to chemokine-induced cellular activation (Bacon *et al.,* 1995). Recent studies have also shown that RANTES may increase attachment of HIV-1 to target cells via glycosaminoglycans and also activate a signal transduction pathway that enhances viral infectivity (Trkola *et al.,* 1999). It is important to recognize that RANTES may exert a complex set of positive and negative influences on HIV-1 replication.

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CCR5 MAY FACILITATE SIV INFECTION

R. Y. Chuang, T. Miyagi, L. F. Chuang, and R. H. Doi

University of California, School of Medicine, Davis, CA

INTRODUCTION

All HIV-1 strains studied to date use CCR5, CXCR4, or both receptors to enter cells, and individuals who lack CCR5 are highly resistant to virus infection (Lee and Montaner, 1999). In addition, a 32-nucleotide deletion within the CCR5 gene has been described in subjects who remain uninfected despite extensive exposure to HIV-1 (Huang *et al.* 1996). SIV infection of non-human primates has served as a useful model for understanding AIDS pathogenesis in humans (Johnson and Hirsch 1992; Chuang *et al.,* 1997b). Research on several genetically divergent SIV isolates have revealed that most forms of SIV, whether macrophage-tropic or T-cell tropic, syncytium-forming or non-syncytium forming, require CCR5 for entry (Chen *et al.,* 1997). CXCR4, on the other hand, does not facilitate entry of any of the simian viruses (Chen *et al.,* 1997). CEM x174, a human lymphoid cell line (Salter *et al.,* 1985), has been routinely used to cultivate and maintain various SIV strains. However, questions have arisen about how CEM x174, which has been reported to be unable to express detectable amounts of CCR5 transcripts (Chen *et al.,* 1997; 1998; Vodicka *et al.,* 1997), efficiently supports the growth of SIV. In searching for an answer, we resorted to a sensitive competitive RT/PCR procedure using primer pairs specific for CCR5 genes, in order to attempt to detect as well as quantify the amount of CCR5 receptors expressed in CEM x174 cells.

RESULTS AND DISCUSSION

In a previous study to quantify virus production in CEM x174 cells, we found that addition of morphine sulfate to CEM x174 cell cultures significantly increases the replication of SIVmac239 (Chuang *et al.,* 1993b). To determine which coreceptor is responsible for the observed morphine effect, we first undertook the task of determining coreceptor densities on CEM x174 cells. Plasmids containing segments of CCR5, BOB (Deng *et al.,* 1997) and BONZO (Deng *et al.,* 1997), and plasmids containing CCR5, BOB and BONZO segments with 96 bp (CCR5), 63 bp (BOB) and 125 bp (BONZO) deletions were constructed. Plasmids with deleted segments were used in quantitative RT/PCR as external controls for quantifying the expression of chemokine receptor genes in CEM x174. The results showed that in addition to BOB and BONZO, CEM x174 cells indeed express CCR5 at the levels of fg quantity per μg of total cellular RNA.

To further establish that the CCR5 transcripts detected in CEM x174 cells are translated into receptor proteins, we performed both flow cytometry and western blot analysis using fluorescein-conjugated mouse monoclonal anti-human CCR5 (for flow cytometry) and rabbit polyclonal anti-human CCR5 (for western blot). Both procedures confirmed the presence of CCR5 molecules on CEM x174 cells.

To investigate the effect of morphine treatment on the gene expression of CCR5, BOB and BONZO, the amount of cDNA amplified by competitive RT-PCR from cells treated with morphine sulfate was compared with that of untreated cells. Morphine treatment, if used, was either 10 μM or 10 nM; these are physiological morphine concentrations in morphine-dependent individuals (Liu *et al.,* 1992). Samples were taken 0, 12, 24, 36, 48, 60 and 72 hr after morphine treatment for analysis. It was found that at 12 hr post-treatment, 10 μM morphine increased CCR5 expression 207% whereas 10 nM morphine induces a 240% increase of CCR5 by 24 hr post-morphine treatment. On the contrary, morphine treatment did not affect the expression of BOB or BONZO in CEM x174 cells. Further experiments showed that the morphine-induced increase in CCR5 expression correlated with the amount of CCR5 proteins on the cell surface and that the effect was opioid receptor-mediated, since it could be completely abolished when cells were pre-treated with naloxone, a *mu* opioid receptor antagonist.

Immune cells have been shown to express brain-like opioid receptors (Chuang *et al.,* 1994; Chuang *et al.,* 1995a; 1995b). Similar to chemokine receptors, opioid receptors are also G-protein coupled, seven-transmembrane domain receptors (Law and Loh, 1999). Human CEM x174 lymphocytes possess all three types of opioid receptors, *mu*, *kappa*, and *delta* (Chuang *et al.,* 1994; Chuang *et al.,* 1995a; 1995b). The current study shows that activation of opioid receptors, probably of the *mu* subtype, by morphine up-regulates the expression of the chemokine receptor CCR5. The downstream molecular mechanisms induced by receptor activation through which morphine affects CCR5 expression awaits further investigation. Morphine, nevertheless, does not perturb chemokine binding to

CCR5 (Grimm *et al.,* 1998). It is therefore attractive to propose that morphine, by binding to its own cell surface receptor, initiates a series of G protein-coupled signal transduction pathways (Chuang *et al.,* 1997a) which thereby hetero-sensitizes (or up-regulates) CCR5. Many facts support this proposal. For instance, morphine has been shown to modulate the expression of other cellular proteins which may induce CCR5 expression. Specifically, morphine reportedly modulates the cellular activation of NFκB and TNF-α in macrophages (Roy *et al.,* 1998) and IL-2 in lymphocytes (Chuang *et al.,* 1993c); activation of NFκB, TNF-α and IL-2 has been found to up-regulate CCR5 expression (Lee and Montaner 1999; Fraziano *et al.,* 1999).

The above studies show that in cells with low levels of CCR5 expression, morphine treatment may bring CCR5 concentrations above threshold levels for maximal infection. In this regard, the induction of the chemokine receptor CCR5 gene expression by morphine may provide a mechanism by which morphine sulfate enhances HIV/SIV infection and hence exacerbates the SAIDS/AIDS pathogenesis.

IMPACT OF COCAINE ON CHEMOKINE RECEPTOR-DRIVEN HIV NEUROINVASION

*S. L. Chang and M. Fiala**

Seton Hall University, South Orange, NJ and *UCLA School of Medicine, Los Angeles, CA

INTRODUCTION

Chronic exposure to cocaine has been shown to increase the adhesion of leukocytes to the endotheliuin, and this effect seems to be mediated via up-regulation of adhesion molecules in the endothelial cells (Chang *et al.,* 2000). Leukocyte adhesion to the endothelial cells is the initial step in the subsequent transcellular penetration of leukocytes across the microvascular endothelial barrier. We have used primary cultures of human brain microvascular endothelial cells (hBMVEC) to explore both the molecular and cellular mechanisms underlying cocaine's effects on the neuroinvasion of HIV-1. An *in vitro* blood brain barrier (BBB) model was constructed (Fiala *et al.,* 1997) to examine the involvement of chemokine receptors expressed on brain endothelial cells.

RESULTS AND DISCUSSION

One of the many functions of the BBB is to inhibit the invasion of micropathogens from the blood into the brain. The BBB is effective at inhibiting penetration of most viruses. However, ample evidence has shown that the BBB fails to stop the invasion of HIV-1. The barrier against HIV-1 in our BBB model was preserved for approximately 1-4 hours post-exposure to HIV-1. Pre-treatment of this *in vitro* BBB model with TNF-α significantly enhanced the viral invasion into the lower chamber in a dose-dependent manner. After 48 hours post-exposure, the degree of HIV-1 invasion in the BBB model without TNF-α was the same as that seen in the model treated with TNF-α, suggesting that the barrier's maximal effectiveness may decline following prolonged exposure to HIV-1, regardless of the presence or absence of TNF-α (Fiala *et al.,* 1997).

The vascular endothelium, characterized by impermeable inter-endothelial tight junctions, provides a crucial interface between the blood and the underlying organ tissue environment. There are at least two major mechanisms of transport: (1) paracellular transport through the intercellular tight junctions of the cells, and (2) transcellular transport via intracellular penetration (Hoek, 1992). Inulin, a well-characterized marker for paracellular transport, was used to investigate the possible mechanism of HIV-1 transport. Both TNF-α and cocaine increased the permeability coefficient of [^{14}C]-inulin across the BBB model. In addition, in a parallel study, TNF-α was shown to increase HIV penetration across the BBB model in a dose-dependent fashion. Cocaine's (10 μM) effect on invasion of the JR-FL strain of HIV in 24 hours was comparable to 100 ng/ml TNF-α. These data indicated that both TNF-α and cocaine enhance HIV penetration across the BBB, possibly through paracellular transport mechanisms, suggesting that paracellular transport is one of the mechanisms underlying HIV's penetration of the BBB. However, there was no difference in the permeability coefficient of [^{14}C]-inulin in BMVEC with and without HIV-1 infection, even though HIV's penetration of the BBB model did increase dramatically. The increase in viral penetration across the untreated model at 24-48 hours post-infection is not accompanied by any change in the paracellular transport of the model. Therefore, HIV-1 penetration across the BBB appears to take place by another mechanism, possibly the transcellular pathway.

These studies then led us to investigate if there were HIV-1 binding sites, including CD4 and chemokine receptors, on endothelial cells that would allow HIV-1 to take a transcellular route across the BBB. CD-4 is an HIV-1 receptor, and certain chemokine receptors have been shown to function as HIV-1 co-receptors on target cells. The expression of the CD-4 receptor on BMVEC was demonstrated using immunocytochemical fluorescence staining.

In addition, expression of some HIV-1 co-receptors on the endothelial cells was also identified (Berger *et al.,* 1999). The immunoreactivity of the CCR3, CXCR4, and CCR5 receptors on the vascular endothelium lining of the brain's blood vessels was demonstrated. The degree of intensity was CCR3>CCR5>CXCR4. Confocal microscopy identified weak expression of CCR5 and strong expression of CXCR4 in cultures of endothelial cells. Using flow cytometry, all three HIV-1 co-receptors were identified on BMVEC, with CCR3 and CXCR4 at high densities and CCR5 at low density. Human coronary artery endothelial cells (CAEC) also expressed these chemokine receptors, whereas expression of HIV-1 co-receptors was not detected in human umbilical endothelial cells (HUVEC).

With these receptors present on endothelial cells, HIV-1 could then be expected to interact with these receptors for efficient entry into these cells. However, following exposure to various HIV-1 strains, BMVEC showed no viral RNA release from the cells for up to 12 days post-exposure. This suggests that, although the BMVEC may have been infected by HIV-1 exposure, these cells do not support the effective replication and subsequent release of the virus post-infection.

We then asked what functional roles these chemokine receptors may play in these endothelial cells, and what is cocaine's involvement? In several parallel studies, cocaine was shown to enhance the secretion of IL-8 and IL-10 and to induce CC chemokine induction in peripheral monocytes. In addition, these chemokines were shown to stimulate the migration of endothelial cells across a neuropore chamber filter in response to a chemokine gradient, resulting in the remodeling of the endothelium. These data suggest that the HIV co-receptors, CCR3 and CXCR4, are present and functional on the brain BMVEC. Although the data showing HIV-1 infection of BMVEC is not conclusive, cocaine's induction of the CXC and CC chemokines in peripheral monocytes suggests that cocaine may have an impact on HIV-1 neuroinvasion, possibly via endothelial cell migration, resulting in endothelium remodeling.

Cocaine was also shown to induce apoptosis in BMVEC cultures, using both an ELISA assay of generated nucleosomes, and the TUNEL assay, which labels nicked-end DNA fragments characteristic of apoptotic cells. Cocaine (10^{-6} to 10^{-4} M) induced DNA fragmentation in a dose-dependent manner. Actinomycin D, which is known to induce apoptosis of BMVEC, served as a positive control.

In summary, although both the HIV-1 receptor, CD-4, and the chemokine receptors, CRC and CXC, which function as HIV-1 co-receptors, are expressed on endothelial cells, including brain microvascular endothelial cells, they may not be involved in the direct invasion of HIV-1. Cocaine increased paracellular transport via tight junctions in BMVEC, and induced apoptosis of BMVEC. In addition, cocaine increased induction of chemokines in peripheral monocytes, chemokines, which could act via chemokine receptors on BMVEC, resulting in endothelium remodeling. Taken together, these findings suggest that the effects of cocaine on the neuroinvasion of HIV-1 may have multiple aspects.

IMMUNOMODULATORY ROLES OF HEROIN AND HIV PROTEIN ON NITRIC OXIDE PRODUCTION BY BRAIN MICROVASCULAR ENDOTHELIAL CELLS

M. P. N. Nair, S. Mahajan, R. Chawda and S. A. Schwartz

State University of New York at Buffalo, Buffalo General Hospital, Buffalo, NY

INTRODUCTION

Over the last several years our laboratory has focused on the molecular mechanisms underlying the effects of HIV proteins on both the immune and central nervous systems. Earlier epidemiological data showed that drug users were at increased risk of AIDS (Center for Disease Control Task Force on Acquired Immune Deficiency Syndrome 1981). Thus we examined the effects of prolonged IV drug use in the context of patients presenting with the prodromal phase of AIDS and compared them with age and sex matched healthy controls. We were the first to

report that intravenous drug users that include heroin and morphine users, manifest depressed cellular cytotoxic activities in their peripheral blood and have elevated levels of soluble serum immune suppressor factor. IFN_ treatment of lymphocytes from drug users with very low NK activity significantly enhanced their cytotoxic activities in contrast to lymphocytes from patients with near normal NK activity. Patients' sera from IV drug users which contained negligible levels of IFN, significantly inhibited the NK and ADCC activities of normal allogeneic lymphocytes (Nair *et al.,* 1986). Our studies were among the earliest to demonstrate that IV drug abuse may be a cofactor for AIDS and that IFNαhad therapeutic potential for HIV infections. Although use of heroin has been associated with immunodeficiency and CNS pathology, the mechanisms of heroin induced neuropathogenesis have not clearly elucidated. Earlier studies have shown that endothelial architecture is modulated during HIV infection and a number of neurotoxic products are known to contribute to endothelial dysfunctions. We hypothesize that although nitric oxide is shown to exert some anti-HIV effects (Chen *et al.,* 1999; Persichini *et al.,* 1999), increased production of NO may cause endothelial dysfunctions and degeneration promoting transendothelial migration of infected monocytes/macrophages and subsequent encephalopathy.

RESULTS AND DISCUSSION

In these experiments we examined the effect of heroin and gp-120 on NO production by Brain Microvascular endothelial cells (BMVEC) and further investigated whether heroin in synergy with gp-120 enhances the production of NO by BMVEC. BMVEC were cultured with different concentrations of heroin alone or gp-120 alone or heroin plus gp-120 for 24 hr and the culture supernatants were quantitated for nitric oxide by ELISA. As a positive control, BMVEC cultured with 10 μg/ml of LPS showed substantial NO production (57.3 μM/L $P<0.01$) compared to untreated control cultures (3.4 μM/L). Heroin at 10^{-6}, 10^{-9} and 10^{-12}M produced 11.1 ($P<0.035$), 13.7 ($P<0.035$) and 13.4 ($P<0.035$) μM/L of NO respectively compared to 3.4 μM/L produced by untreated control culture. BMVEC cultured with gp-120 alone at 1 and 10 ng/ml produced 2.8 and 9.3 μM/L of NO respectively compared to 3.4 μM/L produced by untreated control culture. However when heroin (10^{-6}M) and gp-120 (10 ng/ml) were used in combination, the production of NO by BMVEC was significantly elevated (45 μM/L $P<0.01$) compared to an additive value (20.4 μM/L) of NO produced by treatment with heroin (11.1 μM/L) and gp-120 (9.3 μM/L) alone treated cultures. Heroin (10^{-6} M) and gp-120 at lower concentration (1 ng/ml) did not show any significant synergistic effect on NO production. These results suggest that heroin synergizes with HIV gene products to induce elevated levels of NO by BMVEC to cause endothelial dysfunction, neurotoxicity and associated encephalopathy.

MORPHINE'S INFLUENCE ON AIDS PROGRESSION IN THE MONKEY MODEL

R. M. Donahoe

Emory University, Atlanta, GA

INTRODUCTION

AIDS remains a devastating public health crisis for the U.S.A. and throughout the world with little hope of effective abatement in the foreseeable future. Substance abuse continues to be a major etiological component of the AIDS syndrome, particularly in the U.S.A; and opiates are one of the primary injectable drugs associated with the AIDS milieu. Over the past 2 decades there has been considerable study of the immunological effects of opiates (reviewed in special issue of *J. Neuroimmunol.,* edited by Sharp, 1998). Using animal models and humans, and both *in vivo* and *in vitro* techniques, numerous reports have shown that opiates can modulate many aspects of host immunity. These reports have served as the theoretical foundation for the notion that opiates may modulate susceptibility to AIDS as well as its course of progression. Adding to this notion is the fact that morphine has also been shown to modulate HIV expression *in vitro.*

The question of whether opiate abuse can modify AIDS progression has been examined epidemiologically in numerous studies (reviewed by Donahoe and Vlahov, 1998). Although early literature in this regard suggested that opiates might exacerbate the course of AIDS, the majority of studies since the late 1980s have reported an inability to link opiate abuse with AIDS progression. An exception of note in this regard relates to data reported by Bell *et al.,* (1997) and Davies *et al.,* (1998), that neuropathology of AIDS is more advanced in opiate abusers in comparison with homosexuals. Complicating matters further, several epidemiological studies have reported that opiate abuse retards AIDS progression (see Donahoe and Vlahov, 1998). Two separate animal-model studies using

rhesus macaques to address this issue have also reached opposing conclusions, though these studies are not very definitive because of their pilot nature. Chuang *et al.,* (1993a), reported apparent exacerbation of AIDS by morphine while Donahoe *et al.,* (1993), reported apparent retardation.

Taken together, therefore, the current literature concerning the role of opiates in AIDS is equivocal. This is not to say, however, that it is illogical. In fact, there are a number of circumstances involved with the current studies in this field that support the probable validity of their variable findings given the contextual differences between them. In this regard, it is important to appreciate that opiate effects are adaptable and conditionally variable. For example, consider the monkey data discussed above. When we showed that AIDS progression was retarded by opiate exposure (Donahoe *et al.,* 1993), the opiate exposures involved homeostatically stabilized host physiology. On the other hand, the conditions used in the study of Chuang *et al.,* (1993a), that showed that opiates worsened AIDS progression, were demonstrably less homeostatic. Therefore, it may be that maintenance of homeostatic balance by opiates is 'protective' against progression of AIDS while pharmacological conditions that favor poor homeostasis are 'exacerbatory.' This conclusion makes sense because low stress environments (where homeostasis would be common) have also been associated with slowed progression of AIDS; and, vice versa, high stress environments (poor homeostasis) have been associated with more rapid AIDS progression (Capitanio *et al.*, 1998). Thus, the reason that the outcome of the Chuang paradigm turned out differently than our own may be that the additional stresses of their paradigm promoted AIDS progression. Admittedly, the role of variable viral virulence remains an important unknown in this situation, too, since our studies used a relatively low virulence strain of SIV (SIVsmm9) and the Chuang study used a relatively virulent strain (SIVmac239). Still, it is possible that the variable virulences of these virus strains relate also to their ability to cause variable levels of stress in the host.

The foregoing conclusions are also relevant to the equivocal epidemiological data discussed above. These epidemiological studies were conducted using patients experiencing varying pharmacological and socioeconomic circumstances that are likely to have caused varying levels of stress for the host. In general, poorly maintained opiate dependencies would be expected to cause high levels of stress and well-maintained dependencies would be less stressful. On average, therefore, one might expect the outcome of large studies that contain a broad spectrum of opiate-dependent AIDS positive subjects, as relates to their stress status, to find no significant effects of opiate dependency on AIDS progression. This can only be rectified if subjects can be properly stratified according to their stress levels, a very difficult task.

As mentioned above the majority of epidemiological studies in this area have, in fact, found 'no effect' of opiates on AIDS progression. In this light, it is important to appreciate that there are a number of other important variables that may cloud the outcomes of epidemiological studies, like variations in viral strains and virulence, polydrug abuse, subject-selection bias, and others (Donahoe and Vlahov, 1998). Therefore, it is interesting to note that the one set of epidemiological data that found potential protective effects of opiate abuse on AIDS progression in 'street addicts' (Spijkerman *et al.,* 1996) was done in the Netherlands where opiates are more freely available and their use is likely to be more homeostatic.

RESULTS AND DISCUSSION

It was against the foregoing background that we initiated studies over a year ago to further assess the influence of opiates on AIDS progression in our monkey model that fosters homeostatic drug exposure conditions. We wanted to see if we could confirm our preliminary observations of 'protective' effects against AIDS progression and extend analyses of the reasons for these effects. We now have accumulated data from about 6 months after exposure to both drugs and SIVsmm9 virus, which should still be regarded as preliminary. There have been four major areas of note so far that indicate that opiates are affecting the course of AIDS in the monkeys on study: 1. Mean viral titers appear to be lower in the opiate-dependent monkeys which fits with our hypothesis that our conditions of opiate exposure will retard progression of AIDS. This is so because viral titers ('load') and AIDS progression are known to be strongly positively correlated; 2. Correlations between plasma viral load and absolute CD4 T-cell counts have declined in control animals, from positive to negative over the 6-month course of observation, as expected, while this correlative trend has not been seen in the opiate-exposed group where no obvious correlations exist. These data also suggest that opiates are modulating the viral infection; 3. We have noted several instances of acute immune hypersensitivity that developed at the site of reagent injections in monkeys receiving both opiates and SIV (not saline and SIV) that suggest that regulation of immune balance is being affected by the interaction of SIV with opiates on the immune system. This hypersensitivity is of the Arthus type and seems to be in response to opiate itself, probably because opiates have assumed the role of a hapten, selectively, in the animals involved. Importantly,

many other studies indicate that opiates or SIV alone should not be expected to cause such hypersensitivity; 4. There appears to be an elevated inflammatory incursion of the cerebral spinal fluid in monkeys receiving both opiates and SIV compared with controls. This finding may relate to the observations discussed previously (Bell *et al.,* 1996; Davies *et al.,* 1997) that the course of neuro-AIDS may be altered by opiates.

Obviously, this ongoing study needs to be continued to verify the trends noted so far. Nonetheless, there is every reason to believe that opiate exposure is having an impact on expression of SIV. Given that the group sizes in this study are 18 monkeys each, the observations reported here are very likely to attain statistical significance as the longitudinal observations continue. Continued study to analyze a variety of immunological, pharmacological and virological parameters over time should be very helpful in determining, not only whether, but why opiates have these effects. Though the present study represents a mere beginning in clarifying the role of opiates in AIDS, the present findings are enticing from a clinical perspective as well. If we establish that maintenance of homeostasis by opiates is protective against AIDS progression, there would be reason to suggest that methadone therapy may be beneficial in treating certain addicts with AIDS. Even more, these data suggest that any therapy with the potential to maintain homeostasis, be it pharmacological or psychological, may be beneficial for both drug-dependent AIDS patients and those where drugs are not involved.

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SYMPOSIUM XIII

THE DETERMINANTS OF DRUG DEPENDENCE: CASUAL STATUS OF DIFFERENT ANALYTICAL LEVELS

W. K. Bickel

Department of Psychiatry, University of Vermont, Burlington, VT

Introduction and Overview of Causal Analysis

As the field of drug abuse evolves, our scientific endeavors have become increasingly more specialized and with that specialization, the work of our colleagues in a sub-discipline may become less accessible, and perhaps less understandable to the rest of us. This fragmentation is evident in the growing number of scientific organizations and journals focused on a single analytical level or to only one drug of dependence. Such fragmentation may result in a lack of communication and may lead to an inability to see both commonalties in the problems of different sub-disciplines and how research findings at one level of analysis may influence and impact interpretation at another level of analysis.

Today in this symposium, we focus our attention on closing the gaps in communication and in understanding our colleagues by bringing together leaders in the field whom examine drug abuse from different analytical levels. More specifically, this crosscutting symposium will provide a survey of how different levels of analysis (e.g., genetics, neuroscience, behavioral science, and social science) are thought to be causally related to the problem of drug dependence. To facilitate communication, each speaker was asked to characterize the causal status of their analytical level with the traditional distinctions of causal analysis (e.g., sufficient Vs. necessary Vs. contributory causes: proximal Vs. ultimate) using empirical examples.

To facilitate this conversion, below I briefly review these traditional distinctions in causal analysis (see, Kiesler, 1999, and Mayr, 1988, for a review):

A **sufficient** cause is one that, if present, assures that a particular disorder will occur. Whenever the condition is present, the disorder will occur. A sufficient cause assumes mono-causation. For example, if you are infected with syphilis spirochete, you will have the manifestations of that disease (Kiesler, 1999).
A **necessary** cause is one that must be present for a disorder to occur. This cause must be present when the disorder is present. A necessary cause assumes multiple causation. For example, expression of the dormant tuberculosis bacillus requires mediating factors such as a compromised immune system (Kiesler, 1999).
A **contributory** cause is one that if present makes it more likely that a disorder will occur. Such causes are neither necessary nor sufficient and are typically called a risk factor. A contributory cause assumes multiple causation. For example, smoking is a risk factor for lung cancer as evidenced by the fact that not all smokers get cancer, not all lung-cancer victims are smokers (Kiesler, 1999).
A **proximal** cause ("how" questions) are the mechanisms that result in a disorder and describe the immediate sequence of events that leads to the disorder. Proximal causes assume multiple causation. For example, sickle cell anemia occurs in homozygous carriers of the gene (also, a sufficient cause) (Mayr, 1988).
An **ultimate** cause ("why" questions) describes how the mechanism leading to the disorder evolved. Ultimate causes assume multiple causation. For example, heterozygous carriers of the gene for sickle cell anemia resist malaria more so than those without that gene. Thus, heterozygous carriers have reproductive advantage (Mayr, 1988).

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GENETIC THEORY OF DRUG ABUSE

R. W. Pickens

Virginia Commonwealth University, Richmond VA

Genes are pieces of DNA that contain the information for making a living organism. The DNA sequence is over 99% the same in every human, and this sameness contributes to our basic similarity in biological functioning. Less than 1% of DNA is responsible for genetic variations in humans, but it is this small variation that is believed to be responsible for the development of a large number of diseases and behavioral traits, including drug abuse.

Everyone may be at some risk for drug abuse due to a substance's pharmacological effect on normal reinforcement functioning. For example, "wild type" animals will typically intravenously self-administer cocaine without special environmental conditions. However, some individuals are at greater risk than others to abuse drugs due to genetic and environmental vulnerabilities they may possess. The evidence for this comes from both animal and human studies. In animal studies, animals can be bred to show increased susceptibility to self-administer drugs, and environmental events can be arranged to increase the likelihood of drug self-administration.

In humans, both adoption and twin studies highlight the importance of genetic influences in drug abuse. Adoption studies have found that adoptees with biological parents who abuse drugs, are also more likely to become drug abusers themselves, even when raised by adoptive parents who do not abuse drugs. Perhaps the clearest evidence comes from comparing the correlations for drug abuse in monozygotic (MZ) and dizygotic (DZ) twins. In general, if one member of a MZ twin pair abuses drugs, the co-twin of the pair is significantly more likely to do so as well, compared to DZ twins. Since MZ twins share 100% of their genes while DZ twins share 50% of their segregating genes on average, the greater similarity found among MZ twins is attributed to their greater genetic similarity. Twin studies employing research diagnostic criteria first provided evidence of genetic influences in drug abuse only as recently as 1991, and has been replicated in many studies since then.

In its simplest form, the genetic theory of drug abuse suggests some individuals are born with genetic variations in their DNA that increase their tendency to abuse drugs. An appropriate analogy would be that of a drug-using individual stepping onto a "slippery slope", with genetic influences determining the slipperiness of the slope. In reality, however, the situation is much more complex. For example, genes in drug abuse cannot operate without an environmental substrate. Some environments foster the expression of genetic influences while others hinder their expression. Some environments (e.g., drug availability) are absolutely necessary for drug abuse susceptibility genes to express, regardless of the number of susceptibility genes the person may possess.

Also it is clear that the term "drug abuse" actually encompasses a number of distinctive phenotypes. Typically, these can be distinguished on a continuum ranging from any illicit drug use up to and including the clinical categories of drug abuse and drug dependence. The genetic (and environmental) factors that contribute to drug use, abuse, and dependence may show both common and unique features. For example, the factors that contribute to illicit drug use may be incorporated in the factors that contribute to drug dependence but factors that contribute to drug dependence may include additional genetic influences that do not necessarily contribute to illicit drug use. Research on the genetics of drug abuse is also confounded by secular changes in the clinical definitions of drug abuse and drug dependence, where, for example, the diagnostic criteria for dependence may change over time.

Further, genes may express differently over a person's lifespan. For example, the genes contributing to adolescent drug abuse may be somewhat different than those contributing to adult-onset drug abuse. Gender differences may also be important where the genes contributing to drug abuse in males and females may not only be somewhat different, but the same genes may have different influences in males and females. Recent evidence suggests that both common and unique genetic factors contribute to drug use in males and females. Other factors may influence gene expression in drug abuse as well, such as gene-environment interactions and gene-environment correlations.

At present we know genetic influences are involved in drug abuse, and that the extent of the genetic influence is substantial. Current estimates are that 40-60% of the variance in drug abuse is due to genetic influences. Unfortunately, at the present time our knowledge about the specific genes involved in drug abuse is extremely limited. Lacking this knowledge, we can only speak about genetic influences at the population level. At the

individual level (where prevention efforts can be most important) we currently cannot tell who has a genetic vulnerability to drug abuse.

It is not at all certain that the genes involved in drug abuse will ever be identified. If there are many such genes and each exerts only a very small effect, our current methods of gene identification are not expected to be powerful enough to detect such effects. However, if a relatively small number of genes are involved, or a few genes exert major effects, it will be likely that these genes will be identified in the near future. If we are able to identify specific genes involved in drug abuse, this will raise a disturbing panoply of social and ethical issues. These issues primarily concern who will have access to this information and how it will be used (e.g., job/ insurance discrimination). Given the rapid advances occurring in the field of human genetics, now is the time to start seriously considering possible social and ethical issues as they relate to drug abuse.

Obviously, having the genes for drug abuse does not mean an individual is destined to become a drug abuser. Environmental factors exert a major influence in the etiology of drug abuse, and these factors may be used to ameliorate the effects of genes that may predispose an individual to abuse drugs. Individuals with the genes should not view their situation as hopeless. There are many common examples of successful treatments (e.g., wearing eyeglasses) and preventions (e.g., wearing sunscreen, exercise, changing diets) that have been developed to overcome genetic predispositions to diseases.

THE DETERMINANTS OF DRUG DEPENDENCE: CAUSAL STATUS OF DIFFERENT ANALYTICAL LEVELS – NEURAL EVENTS AS A CAUSE OF DRUG DEPENDENCE

M. J. Kreek

The Laboratory of the Biology of Addictive Diseases, The Rockefeller University, New York, NY

The plasticity of the nervous system following chronic exposure to short-acting drugs of abuse leads to changes in molecular events, cellular events, systems events and in fact overall functioning and behavior. The plasticity involves many processes, including those of learning and memory, which probably implies new synapse formation and consolidation. However, these changes in the brain may be persistent alterations, which remain long after cessation of exposure to a drug of abuse, but are not *a priori* causes of addiction.

One must look to three domains of different factors, including potentially genetic factors, which exist *a priori*; early environmental changes in the brain which may have been induced by pre-natal or post-natal environment; as well as current factors affecting behavior including "set and setting", presence of other diseases, and altered stress responsivity, which may similarly be acquired, or is present on a genetic basis. However, all of these different types of vulnerabilities to developing addictions demand exposure to the drug of abuse before patterns of use, abuse and addiction can occur. Thus, in a causal analysis, one can consider "sufficient" versus "necessary" versus "contributory" components of causes.

Clearly, "necessary" includes availability of and exposure to, usually by self-administration, a specific type of drug of abuse for development of a specific type of drug abuse, dependence or addiction to occur. Both illicit and licit drugs are widely available in the United States and worldwide at this time. It is estimated that over 150 million persons have exposed themselves to alcohol and around 15 million are alcoholic; over 26 million have exposed themselves to cocaine, and one to two million meet the criteria for cocaine dependence; and approximately three million have self-exposed themselves to the illicit short-acting opiate heroin, and about one million meet the dependence criteria of addiction.

As we hypothesized many years ago, and as studies increasingly support, there appear to be three different domains of factors that contribute to the vulnerability and neurobiological (or "metabolic") basis of addiction. These include genetic factors, probably involving multiple alleles of multiple genes, acting in concert to enhance vulnerability. Secondly, drugs of abuse (first hypothesized and now documented) may be able to significantly alter molecular, cellular, neurochemical, physiological, and behavioral events. Finally, there are a variety of host-response factors, ranging from early environment to "set and setting" and presence of other diseases. Exposure to the drug of abuse is essential to unmask any of these domains of vulnerability. Again, considering "sufficient" versus "necessary" versus "contributing" causes, the "contributing" factors could be considered to include any genetic factors

contributing to vulnerability to develop an addiction (or alternatively, protection from an addiction); drug-induced molecular, cellular, neurochemical, physiological and behavioral changes, which may be short-lived, persistent or even permanent; and also diverse early and current environmental factors, behavioral factors and individual "host response" factors.

Further considering "sufficient" versus "necessary" versus "contributing" causes, the "proximal" causes may be considered to include repeated exposure to a specific type of potential drug of abuse (usually by self-administration) for the development of a specific of type of drug abuse, dependence, or addiction. In human drug abusers, it has been shown that as abuse progresses to addiction during use of short-acting opiates, primarily heroin, the pattern of use changes from an occasional exposure, to daily self-administrations of multiple doses of the short-acting opiate, usually heroin, and that the dosing interval between these doses rapidly becomes remarkably regular, both to prevent the onset of opiate withdrawal symptoms, which will occur after tolerance and physical dependence has developed, and also to insure a euphorigenic effect from each subsequent self-administered dose of heroin. In contrast, as humans progress from sporadic cocaine use to regular use, the pattern also changes, but in a different way, usually to one of a "binge pattern", with multiple doses of cocaine administered every fifteen to sixty minutes over a period of two hours or more. Some protracted binges may go on for many hours, followed by abrupt cessation and the onset of a variety of dysphoric symptoms, characterized as a cocaine "crash." Cocaine abuse following this pattern may be on a daily or intermittent basis. Both of these modes, patterns and also routes of administration of heroin and cocaine use have been modeled in animals. When this is done, very different neurobiological findings are made than when these same drugs are administered by pump, which may provide a steady state, or by infrequent depot or pellet administration, which provides a slower onset and offset, but without a steady state established. These findings have shown that the "on/off" effects of a drug of abuse acting at a specific receptor site of action may disrupt molecular, cellular, neurochemical, physiological and behavioral events. In contrast, "steady state" administration, especially as has been well-studied both in animal models and in humans, of an opioid administration (methadone) in steady state, as pertains in appropriate pump method of administration to a rodent, or on daily maintenance of this opioid administration which is long-acting in humans, not only fails to disrupt these events, but allows normalization of that which has been disrupted.

Other "proximal" causes may be considered to include genetic factors. There is possibly the existence, *a priori* (by definition), of multiple allelic variants of multiple genes which may contribute to the development of specific types of drug abuse, dependence or addiction once exposure, usually by self-administration, to a specific type of drug occurs. Over the past six years our laboratory has begun to attempt to identify and then rigorously characterize polymorphisms, especially single nucleotide polymorphisms (SNPs), of specific genes, the expression of which we and others have shown may be disrupted during chronic exposure to drugs of abuse. We have initially focused on the mu-opioid receptor gene and have initially identified SNPs, and more recently have made further identifications. One of these has been studied in collaboration with Lei Yu's group. We have found that β-endorphin is bound three times more tightly to the most common of these variants, and that the G-protein coupled inwardly rectifying potassium currents are also enhanced three-fold following β-endorphin binding to this variant allele, compared with the prototype receptors. However, whether or not this may offer a protection against development of addiction, or enhancement of any function under the mu-opioid receptor modulation, has yet to be determined.

We hypothesized many years ago that atypical responsivity to stress and stressors may contribute to the acquisition, persistence of and relapse to drug abuse. Atypical responsivity to stress and stressors might be on a genetic and/or early environmentally induced basis. Also, certainly, many groups have documented that alterations in stress responsivity can be caused by exposures to drugs of abuse, including heroin and cocaine.

In a further consideration of "proximal" and "ultimate causes", "ultimate causes" could be considered to be any or all of those changes in molecular, cellular and neurobiologic, as well as subsequent physiological and behavior changes which occur following chronic exposure to a drug of abuse and which may contribute to the progression of drug abuse to dependence and addiction, as well as subsequent relapse. Again, many studies have documented such a sequence of events.

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ENVIRONMENTAL FACTORS IN DRUG DEPENDENCE: CAUSAL ANALYSES

J. L. Falk

Dept. of Psychology, Rutgers, The State University, Piscataway, NJ

The pharmacological action of a drug, including its behavioral action, is contextually modifiable as well as conditionable. If a particular drug agent is a necessary, but far from sufficient cause of drug abuse, what sorts of environmental events are responsible for catalyzing the extraordinary behavior we call "drug abuse"? I want to emphasize that this action is not just permissive, not just a matter of facilitating an intrinsic pharmacological determinant. Although I don't want to deny that environmental factors can function simply as contributory risk factors, it is important to realize that the environment also may contain proximal causes that are sufficient for inducing a class of behavioral excesses, including drug abuse. These behavioral excesses have become incorporated by evolutionary processes into the behavior of diverse species because they have served an adaptive function. That is, these conditions produce excessive behavior owing to reasons of ultimate causation. This is not to argue that drug abuse itself is in any way adaptive behavior. But it may be the baleful, dark side of excessive behavior that has been programmed to occur by evolutionary mechanisms because such behaviors often do have adaptive consequences. Drug abuse, excessive aggression, rape, hyperactivity, unwise gambling, etc. may be cul-de-sac side effects of processes that have been strengthened through evolution owing to their originally adaptive consequences.

In several animal species the delivery of small, reinforcing events at an attenuated rate will induce a range of excessive behaviors (e.g., large fluid intakes), as long as the rate of reinforcement is not too extremely attenuated. The same general condition--attenuated food-delivery rates--also can induce excessive attack, hyperactivity, particularly stereotyped and ritualistic activities (Falk, 1971). The same constraints on food delivery can induce animals to ingest heroic amounts of drug solutions, such as ethanol, barbiturates, cocaine, PCP or benzodiazepines, with behaviorally interesting consequences (e.g., severe physical dependence). Animals can easily be induced to prefer drug solutions to a concurrently available water vehicle alternative as long as the inducing conditions remain in effect (Falk, 1998). The general phenomenon is known as schedule-induced, or adjunctive, behavior.

A lack of valued things in addition to food, such as love or money, can also lead to excessive adjunctive behavior in humans. Even a severe lack of a valued commodity is not in itself a sufficient condition to induce adjunctive behavior. It is deprivation in conjunction with the constraint of episodic delivery of limited portions of a crucially valued commodity in one domain that induces excessive behavior in relation to commodities in some other domains. These inducing conditions may be functionally homologous to ones catalyzing drug overindulgence in humans. When life's crucial commodities are in short supply and available only on intermittent, marginal schedules, such as may occur in an impoverished, inner-city environment, drugs can become all-powerful in their reinforcing efficacy.

Situations in which a highly motivated, crucial activity is occurring (e.g., parental nest defense, courtship or mating, or an agonistic encounter from an intrusion), and under which an attenuated rate of reinforcement from the crucial activity is occurring: an intruder is threatening the nest, or eggs have been stolen; a mating sequence cannot be completed owing to threat postures of the mate; territorial holding is endangered by competition or pressure from a predator; a feeding patch may have become depleted--all are situations where the rate of reinforcement for a crucial activity is such that there is ambiguity as to whether the best course of action is to a) remain engaged, or b) disengage (escape, withdraw, migrate, relinquish). Equivocal situations, in which the vectors to "stay-engaged" versus "leave-the-field" are about equal are situations giving rise to what ethologists call "displacement activities"

and what we observe in chronic, experimental situations as adjunctive behavior. There is a strong reason to stay in the situation, but because of marginality, threat, or other negative factors, there is also a strong motivation to escape the situation.

This sort of ambiguity or ambivalence is crucially consequential as it concerns both a) ontogenic survival (e.g., food supply, territory, avoidance of predation) and b) phylogenic survival (e.g., mating, incubation, nest defense). Given crucial consequentiality in a situation of ambiguous utility (an unstable equilibrium), any movement toward continued engagement or disengagement will perpetuate and will probably be of fateful significance--the individual decisively remains engaged or leaves. It is best, in terms of both individual and species survival, if such fateful decisions are based on adequate situational information as to whether the best course of action is to remain engaged or move on. Otherwise, the unstable equilibrium might be fortuitously tipped in the wrong direction for survival--i.e., stays engaged when best to have left, or leaves when it would have been best to stay. If the resolution of the ambiguous situation can be delayed until more fine-grained information becomes evident, then the decision is likely to be more advantageous.

The best way to put resolution of the situation "on hold" is to diversify behavioral output in a way that occludes escape from the situation until the ambiguity of the competing vectors, "the conflict," has a chance to be clarified. That is, with more time to sample the situation, for more information on it to be acquired, the utility of staying or leaving is more likely to become clear. The holding action effected by displacement activities will result in a better-informed decision on engagement versus disengagement.

The advantageous survival consequences of strong behavioral diversification is the ultimate cause underlying the generation of adjunctive behavior. Strongly "doing something else" by coming under the stimulus control of local alternatives is a process that delays precipitous commitment until the situation producing the opposing vectors becomes clearer as to its rate of reinforcement and overall value (Falk 1977).

In society, individuals can be locked into a marginal subcultural setting, a ghetto or even an exclusive academy, affording little escape opportunity, in which crucial conflicting vectors are permanent features. Diversifying into another behavior in such a circumstance leaves the conflict unchanged--it does not, as in ethological encounters, simply allow time for clarification and resolution of the situation. Rather, the predicament is likely to be an endemic one. One can conceive of society as a kind of chronically constraining environment. Often, there are continuing conflicts one faces for years on end: acceptance of the status quo versus revolution; duty and honor versus passion; work routines versus avoidance of onerous tasks; child-rearing versus free-ranging careerism; conservation of resources versus creative risk-taking. Unlike ethological encounters, these oppositions are not acute situations which will resolve definitively one way or the other. They are all too often continuing conflicts, concerned with crucially important life issues, and they generate streams of adjunctive behavior from benign to creative to toxic. One of the toxic ones is the chronically intrusive behavior known as drug abuse.

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SELECTED ISSUES OF CAUSAL INFERENCE IN EPIDEMIOLOGICAL RESEARCH

J. C. Anthony

Johns Hopkins University, School of Hygiene and Public Health, Baltimore, MD

Many epidemiologists devote their careers to tasks of basic descriptive surveillance, with no sustained attention to issues of causal inference. Nonetheless, issues of causal inference surface even in the most descriptive work, and issues of causal analysis penetrate the history and practice of epidemiology:

> A great part of clinical medicine, and of epidemiology, must still be observation. Nature makes the experiments, and we watch and understand them if we can. No one will deny that we should always aim at planned intervention and closer control. Here, as elsewhere, technique – the way we make our observations and check them – is half the battle, but to force experiment and observation into sharply separated categories is almost as dangerous a heresy as the science and art antithesis. It tends to make the clinician in the ward, the epidemiologist in the field, and the laboratory worker at his bench, think of themselves as doing different things, and bound by different rules. Actually, they are all making experiments, some good, some bad. It is more difficult to make a good experiment in the ward than in the laboratory, because the conditions are more difficult to control; but there is no other way of gaining knowledge..... Controlled observation in the ward or in the field is an essential part of medical science, shading through almost imperceptible stages of increasing intervention into the fully developed experimental technique of the laboratory. (William Topley, quoted by A.B. Hill, 1953).

One of the earliest lessons of epidemiology was that important evidence to guide the prevention and control of disease can be discovered years and even decades before there is a firm understanding of the ultimate or essential causes of disease, and well before the disease phenotype has been characterized with precision and specificity. This lesson was apparent in the work of John Snow, acknowledged as the British father of epidemiology, who taught the world that relatively simple water sanitation practices can curb and prevent epidemics of cholera. This insight emerged from analyses of quite crude epidemiological data on cholera mortality, during an era when cholera was diagnosed by non-specific clinical methods, and more than a decade before Robert Koch identified *cholera vibrio* as the cause of cholera. The same lesson is found in the work of Joseph Goldberger, sometimes regarded as the American father of epidemiology, who inferred from observational data that pellagra should not be a disease of infectious origin, and whose epidemiological field studies, clinical research, and experiments during the early 1900s confirmed its origin in a matrix of seasonal variation, socioeconomic conditions, and institutional practices that lead to restricted diets. Decades later, long after effective preventive maneuvers had been started, niacin deficiency was identified as a root cause of pellagra (Anthony & Van Etten, 1998)

One difficulty in epidemiological research on cause-effect relationships is that most epidemiological evidence comes from observational studies, as in traditional astronomy, geology, and ecology. Even when it might be logistically feasible to make a randomized assignment of two or more causal conditions, there can be ethical constraints. For example, when AIDS was thought to be a disease secondary to the use of nitrite inhalants (i.e., 'poppers'), no one proposed a randomized trial to test this hypothesis by having one group use poppers with amyl nitrite and another group use poppers with some inert substance.

Rather, in keeping with a tradition that dates back to Koch's postulates in bacteriology, epidemiologists look toward a set of criteria that serve as general guidelines in the search for causes and effective preventive maneuvers, once an association is observed between a disease and a suspected causal influence. The main criteria are as follows: (a) strength of the association, (b) confirmation of the association by replication, (c) quantitative relationship between amount of exposure to suspected influence and subsequent response, (d) chronologic relationships (cause before effect), and (e) biological reasonableness of the association (e.g., see Sartwell, 1960), with some investigators suggesting additional criteria such as specificity of the association, plausibility of the association, coherence of the association, evidence from experiment, and reasoning from analogy (e.g., see Hill, 1965). The criteria for 'biological reasonableness,' 'plausibility,' and 'coherence' stand for a combination of theory and evidence. Whereas a psychologist or sociologist might discuss the adequacy (or inadequacy) of an investigator's conceptual model or theory, an epidemiologist is more inclined to examine the biological reasonableness of the model, the plausibility of a unidirectional causal path from one model element to another versus a reciprocity, and coherence of the model in relation to a composite of observed evidence about related matters.

In this context, space limitations preclude a thorough review of these criteria; interested readers will find suitable appraisals of each criterion in modern textbooks of epidemiology (e.g., Rothman and Greenland, 1998). A careful analysis of these appraisals will disclose that some widely-accepted criteria actually have limited utility. Requirements for a sigmoidal dose-response relationship in the association between exposure and risk of adversity often is ill-advised, and a requirement for specificity of the association is short-sighted (e.g., see Sartwell, 1960).

It seems more important to use the remaining space to draw attention to some neglected issues in relation to statistical aspects of causal inference and to issues that concern samples enrolled in clinical and epidemiological

trials. With respect to statistical aspects of causal inference, it is important to note that many investigators (present company excluded, of course) have not paid attention to development of the likelihood paradigm as an approach to scientific evidence. Instead, they get trapped within a framework of rote-learning about ritualized procedures and conventional but unimaginative thinking about statistical significance, p-values, and corrections for multiple comparisons. These are scientific issues, not matters of dogma; there are alternative points of view that warrant careful study. In this context, Royall's introduction to the likelihood principle and causal inference within a likelihood framework offers a welcome alternative to today's adherence to statistical ritual (Royall, 1997).

With respect to questions about the samples enrolled in clinical and epidemiological trials, it should suffice to say that there are some limits to the utility of estimates based upon recruited volunteers when the investigators pay little or no attention to individuals who refused to participate, did not comply with treatment, or who were not included within the explicit or implicit sampling frames for the studies. On one hand, it is very useful to know that Treatment A or Prevention B causes such and such a reduction among subjects who enroll in a randomized trial and show 100% compliance. This type of information might be of great value to a clinician who treats help-seeking patients, or to a prevention specialist whose charges are volunteers. On the other hand, if the task is to gauge the broad public health impact of a treatment or preventive intervention, then there is reason to ask for an estimate of the intervention's effect in the rest of the population: i.e., those who need treatment but do not seek it, those who are uninterested in participating in a drug prevention exercise.

A conventional approach is to assume that the sample under study is representative of the population at large, and that a reduction of X% of disease burden in the observed sample will generalize to a reduction of X% of disease burden in the general population from which the observed sample was drawn. In actuality, this conventional approach may well yield a distorted view of the benefits of treatment or prevention program participation. Potential subjects who do not agree to be treated, who comply only partially, or to enjoy the benefits of prevention programming might yield substantially different and often lower effect estimates.

In sum, epidemiology often seeks effective preventive maneuvers well before ultimate causes can be inferred or stated with certainty. Epidemiologists have developed a formalized set of criteria for judging the causal significance of observed associations, but the touchstone of evidence remains much as it is appreciated in other branches of clinical medicine and the biomedical sciences. All else being equal, results from randomized experiments generally are evaluated with more weight than results from non-randomized observations. Nonetheless, there are reasons to be cautious about generalization from pools of subjects who are willing to participate in randomized trials, whether these are to evaluate treatments or preventive maneuvers. Two of the challenges for new investigators in the 21st century are to investigate the potential effects of interventions on individuals who do not agree to participate or comply fully in such trials, and to clarify the utility of likelihood principles in causal inference about suspected causal or preventive influence when the pack still turns to ritualized procedures and inexact statistical thinking.

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SYMPOSIUM XIV

THE ALCOHOL AND DRUG SERVICES STUDY (ADSS)

C. M. Horgan

Schneider Institute for Health Policy, Heller Graduate School, Brandeis University

The Alcohol and Drug Services Study (ADSS) was designed to collect detailed national data describing characteristics of substance abuse treatment facilities, clients in treatment, and treatment outcomes. The study gathered information linking program organization, financing, costs, clients, treatment, services, and outcomes. ADSS data represent an important step toward understanding the factors that affect the content and quality of treatment, access to and availability of appropriate care, the relative cost-effectiveness of treatment, and managed care contract arrangements.

PROJECT DESIGN

ADSS encompassed three phases of data collection corresponding to the study of treatment facilities (Phase I), clients in treatment (Phase II) and treatment outcomes (Phase III), and also included several substudies. ADSS is based on a nationally representative sample of alcohol and drug abuse treatment facilities selected from SAMHSA's 1995 National Master Facility Inventory which was augmented to encompass the universe of substance abuse treatment facilities in the United States. Since ADSS is based on sample data, weights have been developed to produce national estimates of facilities and characteristics of clients in treatment. Data were collected between 1997 and 1999. Facility level data collected in Phases I and II, combined with client level data collected in Phases II and III allow for cost-effectiveness analyses, as well as other measures of treatment outcome.

The purpose of Phase I was to provide a description of the substance abuse treatment system at the facility level, reflecting the range of modalities from residential, inpatient, outpatient methadone and outpatient non-methadone. It consisted of a mail survey collected by telephone interview with the director at approximately 2,400 facilities. Data were collected for the point prevalence date of October 1, 1996, and for the most recent twelve month reporting period of the facility. Three types of data were collected: organizational, *e.g.* ownership, services offered, staffing; aggregate client, *e.g.* demographics, special populations, referral sources; and financial, *e.g.* revenues, sources of revenues, managed care.

The purpose of Phase II was to describe the client population and the process of substance abuse treatment. Phase II involved a site visit to a subset of 280 facilities from the Phase I sample. Site visit activities included two components: an interview with the facility administrator to collect information on facility treatment practices and policies and further financial documentation, and sampling and abstracting over 6,000 client records, primarily for discharged clients. The client record abstract obtained data on demographics, other background information such as criminal justice involvement, medical information, substance abuse history, treatment services, discharge information, and sources of payment. A cost substudy collected detailed information related to the cost of providing treatment by constructing an audit spreadsheet and verifying data via telephone interview with facility staff after the site visit. An in-treatment methadone substudy, involving client record abstraction of a sample of approximately 900 clients was conducted in order to compare an in-treatment methadone sample to a discharged methadone sample.

The purpose of Phase III was to describe the post-treatment status of clients. It consisted of an in person interview and accompanying urine testing with approximately 3,000 Phase II clients on average twelve month follow-up. These interviews determined post-treatment status in terms of alcohol and drug use, criminal justice status, psycho-social functioning, further treatment episodes, and other health status. An incentive substudy was also conducted as part of Phase III to study the effect of different levels of incentive payment on response rate, response quality and sample bias. Another substudy on early dropouts was conducted to compare outcomes for clients who were in treatment for no more than one day or one visit to clients who stayed in treatment longer.

DESIGN ISSUES

Major strengths of ADSS include: the large sample size (both of facilities and clients), focus on the full range of treatment modalities, the national representativeness of its design, the ability to link facility and client level data, and the cost data collection strategy. These strengths afford numerous analytic opportunities to better understand the national treatment system and the clients it serves.

In collecting data in large scale, multi-component studies such as ADSS it is important to recognize special design issues that are inherent in collecting this type of data. First, these studies typically do not include all types of specialty treatment, such as treatment within the criminal justice system and office-based practitioners. Second, facility data collection may require multiple respondents with specialized expertise particularly necessary for financial information. Third, in conducting a study in which a facility is to be contacted at two points in time, one must be prepared to deal with substantial director turnover. Fourth, a representative sample involving all types of facilities, versus a purposive sample which may be research oriented, presents special challenges because of the variability in facility record keeping. Fifth, facility issues impact client response rate. These include incomplete client locator information at the facility and director turn-over resulting in new directors who do not want clients to participate in the study. Sixth, tracking clients is difficult because of their mobility. Finally, all surveys, not just substance abuse surveys, need to deal with the temporal phenomenon of lower response rates.

ACKNOWLEDGMENTS: ADSS was supported by the Office of Applied Studies (OAS), Substance Abuse and Mental Health Administration (SAMHSA), U.S. Department of Health and Human Services. It was conducted under contract by Brandeis University. The subcontractor for field data collection was Westat, Inc. Capital Consulting Group collaborated in the cost substudy. Helen Levine was the Phase I and II lead. Mary Ellen Marsden and Christopher Tompkins were the Phase III leads. Don Shepard headed the cost substudy. Margaret Lee was project manager. Grant Ritter was lead statistician for Brandeis. Sharon Reif and Aaron Beaston-Blaakman served as research analysts. The SAMHSA project officer was Anita Gadzuk.

HOW USEFUL ARE LARGE-SCALE EVALUATIONS OF SUBSTANCE ABUSE TREATMENT?

T. D'Aunno

University of Chicago, School of Social Service Administration and Pritzker School of Medicine

Given the amount of resources dedicated to several prominent large-scale evaluations of substance abuse treatment (e.g., Hubbard *et al.*, 1989; Gerstein *et al.*, 1994; Simpson and Curry, 1997; Horgan *et al.*, 1992), and considering weaknesses in these studies (Gerstein and Johnson, 1999), it is important to raise questions about the extent to which they are useful and how future efforts can be improved.

My view is that, in theory, there is a compelling argument to support the conduct of large-scale evaluations of substance abuse treatment. This argument begins by emphasizing that there are many factors that can affect the extent to which a particular treatment approach works in any given practice setting. Key factors include characteristics of clients (e.g., motivation; drug-use patterns), clinical staff (e.g., motivation; training), and programs (e.g., funding levels; managed care arrangements). As a result, to assess the effectiveness of substance abuse treatment as it is actually practiced in community settings, we need to conduct studies that take into account the role that client and program characteristics can play in influencing outcomes. Doing so would not be especially difficult if there were relatively little variation in clients and programs from one setting to the next. Many empirical studies show, however, that there is substantial variation in the nation's treatment providers, treatment practices, and clients (e.g., D'Aunno *et al.*, 1999). This variation means that to assess treatment effectiveness we need studies that are based on: (1) representative samples of a defined population of both treatment providers and their clients; (2) samples of treatment programs and clients that are large enough so that there is adequate statistical power to identify the contributions, if any, of characteristics of clients, treatment programs, and practices to outcomes.

Moreover, there have been significant changes in clients (such as their drug-use patterns) and in the organization and financing of treatment services (such as managed care) that can affect treatment quality and effectiveness. Thus, large-scale assessments of treatment effectiveness should be conducted periodically to determine how changes in

clients and the context of treatment influence treatment outcomes. In the absence of such studies, policy-makers and planners will have great difficulty making rational decisions about how to allocate scarce resources for treatment. Similarly, it will be difficult to identify ways to improve treatment programs and practices without large-scale, comparative evaluations.

Nonetheless, though one can argue that, in theory, large-scale studies are needed, in practice, prior studies have been limited by relatively small sample sizes of programs; samples that were not designed to be widely representative of treatment programs; and relatively low response rates at both the client and program levels of analysis (Gerstein and Johnson, 1999). These limitations make it difficult to generalize results and to assess the contribution of program characteristics to treatment outcomes. In other words, these limitations threaten to undermine the central rationale for conducting large-scale evaluation studies in the drug abuse treatment field.

I argue that, in response, we need to consider some important and specific improvements in the design and implementation of large-scale evaluations. First, research designs need to increase the number of treatment programs included in samples, and such samples need to select programs so that they represent particular populations or sub-populations of treatment providers (e.g., a national sample of methadone treatment providers). Second, analyses of non-response bias should conducted with data that have been collected to determine if low response rates may be biasing results and, if so, how adjustments for non-response might be made. Third, future studies should allocate sufficient resources for data collection so that they can attain high response rates for both clients and programs.

In sum, these changes in the design and conduct of large-scale evaluations would increase their potential to contribute to our understanding of treatment effectiveness. In the absence of such improvements, however, we ought to consider halting large-scale studies so that resources can be used more effectively to address other needs in research and practice.

REFERENCES: Available upon request from the author.

AN UPDATE ON THE DRUG EVALUATION NETWORK SYSTEM (DENS)

A. T. McLellan, D. Carise, and H. D. Kleber

[1]Treatment Research Institute at the University of Pennsylvania and [2]The National Center on Addiction and Substance Abuse at Columbia University

INTRODUCTION

Although an estimated 1 million Americans enter addiction treatments each year, we know very little about this population. In particular, we have no recurring, descriptive information on such basic characteristics as demographics, types and amounts of substances used prior to treatment entry, or the nature and severity of their "addiction related" problems in the areas of medical health, employment, criminal activity, or psychiatric status.
With the support of the Office of National Drug Control Policy, we have designed and initiated the Drug Evaluation Network System (DENS), to collect clinically and policy relevant information directly from patients entering a national sample of addiction treatment programs. This system uses an Addiction Severity Index interview collected on laptop computers and transmitted electronically to a central server on a weekly basis.

Design of the Drug Evaluation Network System (DENS)

Patient Information - The Addiction Severity Index (ASI) is the primary source of patient information in the DENS. The ASI includes information on the nature, number, and severity of drug and alcohol problems, as well as severity of patients' medical, family, legal, employment, and psychiatric needs.

Treatment Information – We decided to limit collection of during -treatment information to modality of treatment, length of stay and type of discharge. These have been well related to treatment outcome from most types of addiction treatment.

The Sample - DENS is being installed in a national sample of 200 programs drawn from the frame of public and private, alcohol and drug treatment facilities listed in the National Master Facility Inventory (NMFI) maintained by SAMHSA.

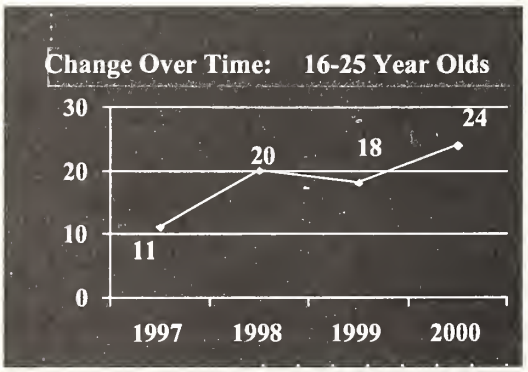
Collection of the Data - DENS programs receive a laptop computer and ASI software packages to collect the data, print out clinically useful reports and transfer information to our central server. All data are automatically screened for errors and inconsistencies during the interviewing process. To have immediate clinical value at the time of admission, DENS software was provided to transform the ASI data into a six page clinical narrative suitable for use as an intake or admission summary, and as a guide to initial treatment planning for each patient. The data collected throughout the week is sent via pre-programmed modem to TRI in a total time of approximately 3 minutes. All programs have complete access to their own database and partial access to the full national data system, to permit comparisons between local and national trends.

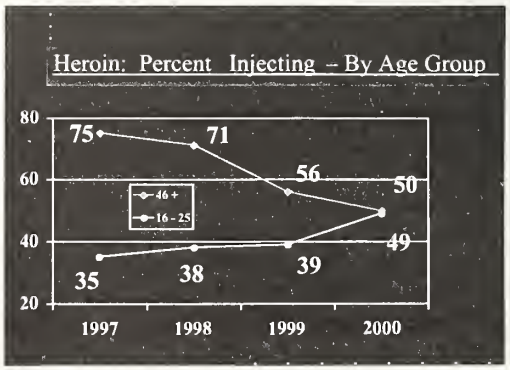
Illustrative Data

There has been question whether there are "new" heroin users entering the treatment system and whether they are at risk for infectious diseases due to route of administration. Data from a one-time sample of "younger" (e.g. 16 – 25 year old) and "older" (e.g. 46 or older) clients who entered the sample of programs during June 2000 are available from the DENS data. They reveal that of all admissions to the system who used heroin in the past 30 days (approximately 2,400 admissions or 18% of all admissions to the system) 24% were in the "young category" and 21% were in the "old" category. Further, we found that within these samples of heroin users 51% of old and 49% of young users had used by injection. These data by themselves suggest that there is a relatively small proportion of young heroin users and that while disturbing, it is at least no greater than has been seen among older users.

In fact, the data from such a simple, one-point-in-time survey are misleading since there is no indication of change. The DENS offers continuing monitoring and trend analysis. Below we present the proportion of young (16 – 25) heroin users as a percentage of all heroin admissions to the system (Figure 1) and the proportion of injection both younger and older heroin users who were injecting opiates at the time of their admission (Figure 2).

As can be seen, the data indicate that the proportion of younger heroin users has more than doubled since 1997. Even worse, while the proportion of injectors has decreased among older users it has actually increased among the younger subgroup. These data combine to suggest the importance of prevention and treatment measures targeted to this subgroup.





After successful piloting of DENS, we are ready to implement a representative national sample of treatment programs. Programs selected will include publicly and privately funded programs from all major treatment modalities. The DENS expansion provides a framework for the design of rapid and cost effective outcome evaluations.

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NATIONAL EVALUATION OF SUBSTANCE ABUSE TREATMENT (NESAT)

H. D. Kleber

National Center on Addiction and Substance Abuse at Columbia University

What is NESAT

The National Evaluation of Substance Abuse Treatment (NESAT) is the first study of substance abuse treatment outcomes that combines a prospective design with a national probability sample of treatment programs and clients receiving treatment in metropolitan areas. This study, which began data collection in August 1997, consisted of baseline data collections from service delivery units (SDUs) in five treatment modalities: Residential Chemical Dependency Units, Therapeutic Communities, Methadone Maintenance Programs, Intensive Outpatient, and Non-intensive Outpatient Programs. Clients entering treatment in those SDU's were to be interviewed shortly after entry, and as well a 30-day follow-up data collection and 12 to 24 month data collections from SDUs and clients. Approximately 164 SDUs and 2,100 clients participated in the baseline data collections. The surveys of SDUs collected information on their treatment programs, staff and client characteristics, and finances. The surveys of clients collected information on their substance abuse treatment history, substance abuse, medical status, employment status, legal status, and psychiatric status: at the 12- to 24- month data collection. NESAT also collected hair, urine, and breath specimens from clients. We were able to interview 72 percent of these 2,100 clients at follow-up (78% of those who were not incarcerated).

Uniqueness of NESAT: Relation to Other Studies

No other national study includes all of the key NESAT elements: prospective design, national probability sample of clients and programs, detailed data on the programs and treatment, and biological outcome markers.

The prospective design of NESAT means that it could collect highly comparable information on clients (e.g., on recent substance abuse and criminal behavior) at multiple points in time. For this reason, comparisons of baseline results and results at subsequent time points have greater power--that is, they are more likely to detect differences across time--than if records-based data alone are used for the baseline.

By using a national probability sample of treated clients, NESAT permits national estimates of known precision. The national representativeness of the study sample sets NESAT apart from other studies that relied on a purposive or non-probability samples of SDUs such as the National Treatment Improvement evaluation Study (NTIES) and the Drug Abuse Treatment Outcome Study (DATOS).

Another strength of NESAT is that it offers detailed information on the SDUs that provided treatment to the clients enrolled in the study and on the treatment that clients received.

NESAT also offers biological specimen data from clients for the 12- to 24-month data collection and achieved high cooperation rates (i.e., clients who provided specimens divided by the number of clients who completed follow-up interviews): 80 percent for urine, 66 percent for hair, and 83 percent for breath. With these data, NESAT researchers can address concerns about the validity of self-report behavior on sensitive topics.

NESAT Limitations

Although the probability sample design for NESAT has definite advantage, it also has limitations in the form of response rates. Because the SDUs were part of a probability sample, they were less cooperative in helping to enroll clients into the study than they might have been if they were selected purposively because they were associated with the survey funding source or with the researchers. (Because of confidentiality requirements, NESAT interviewers relied on SDU staff to link sampled potential study participants with the interviews.) Hence, the NESAT overall response rates (i.e., clients who completed interviews plus ineligible clients divided by the number of clients who were originally sampled) was 54%, lower than DATOS and NTIES. Even with its lower than desired response rates, however, NESAT's design offers advantages over purposive sample studies that are unable to make any claims to national representativeness. However, we are unable to say whether the sample recruited fully represents the population of patients within each of the programs.

An additional limitation of this study concerns the long-term follow-up component of NESAT. While it was originally designed to re-interview clients approximately one year after their enrollment in the study, the long-term follow-up interviews occurred somewhere between 12-and 24-months after enrollment. This unforeseen extension makes it impossible to talk about a one-year follow-up, but the distribution of follow-up interviews over this period will enable us to explore the relationship between client entry-level characteristics and the length of time to obtain the follow-up interview and the relationship between the time elapsed since the baseline interview and key outcome variables such as relapse and re-entry into treatment.

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SUBSTANCE ABUSE TREATMENT OUTCOMES IN THE 1990S: A COMPARISON OF MULTISITE STUDIES

D. R. Gerstein and R. A. Johnson

National Opinion Research Center at the University of Chicago

Research advances on fundamental policy-relevant aspects of substance abuse treatment have arisen from a mixture of small experimental studies and large observational studies. Before the 1990s, large studies—which may be operationally defined as follow-ups of more than 1,000 treated individuals under one protocol—were rare, occurring at roughly once-every-ten-year intervals (Sells, 1974; Hubbard *et al.*, 1989; Simpson and Curry, 1997). However, in the early to middle 1990s, not just one but four large-scale observational studies were performed, with observed outcomes in samples ranging from 1800 to 5400 cases. These studies were close enough in time and design features to permit bringing the results together in a new way. In this analysis we directly compare the major features (research methods, provider and patient characteristics) and outcome results of the four studies for the first time, appraising these data as an unprecedented 300-program/10,000-person observational laboratory of substance abuse treatment follow-up data.

The four studies are the California Drug and Alcohol Treatment Assessment, Services Research Outcomes Study, National Treatment Improvement Evaluation Study, and Drug Abuse Treatment Outcomes Study, which are known by the abbreviations CALDATA, SROS, NTIES, and DATOS (Gerstein *et al.*, 1994; Schildhaus *et al.*, 1998; Gerstein *et al.*, 1997; Simpson and Curry, 1997.) The first two studies were stratified random probability samples of treatment providers and patients at the state (CALDATA: California) and national (SROS) levels using retrospective one-contact designs. NTIES and DATOS were prospective, repeated-interview designs focusing on evaluations of selected urban provider networks with relatively high percentages (60-70% black and hispanic versus ~40% in the fully randomized designs) of minority group clients. Three of the studies covered approximately one-year before-treatment and after-treatment reference periods,and SROS covered five-year reference periods. All studies achieved multistage (multiplicative) response rates of about 60 percent of the estimated eligible study sample (all persons admitted to treatment in the sampled provider units who completed one or more visits or overnight stays, including in the denominator sampled but noncooperating providers as well as patients not successfully recruited to treatment or recruited but lost to followup)—except for DATOS, which achieved about 40 percent.

Pre-Post Percentage Change in Selected Measures of Drug Use and Behavioral Functioning in the CALDATA (C) and NTIES (N) Studies

	Marijuana		"Crack"		Heroin		Arrests		Disability		Employment	
	C	N	C	N	C	N	C	N	C	N	C	N
Short-term resid.	**-53**	**-57**	**-64**	**-53**	**-36**	**-62**	**-67**	**-63**	**-52**	**-12**	**n.s**	**16**
Long-term Resid.	**-51**	**-51**	**-68**	**-60**	**-26**	**-41**	**-68**	**-62**	**-49**		**n.s**	**32**
Outpatient	**-34**	**-42**	**-51**	**-52**	**-35**	**-45**	**-66**	**-60**	**-31**	**n.s.**	**-11**	**18**
Methadone Maint	**-20**	**-42**	**-36**	**-45**	**-39**	**-51**	**-82**	**-55**	**n.s.**	**n.s.**	**n.s.**	**n.s.**
Methadone Disch	**-31**	**-42**	**-20**	**-23**	**-13**	**-25**	**-38**	**-34**	**-21**	**-15**	**-34**	**n.s.**

As illustrated in the table above, which displays data from two of the studies (CALDATA and NTIES), each of the data sets revealed broad positive changes in drug use (measured here as self-reported use 5 times or more during the reference period, validated by urinalysis), crime (self-report of any arrests, validated by criminal records check), and health status (using here a standard global health status or disability self-report scale). Clients in short-term and long-term residential treatment tended to report positive changes in drug use, crime, health, and employment (as measured by holding any full-time job) somewhat more often than clients in outpatient treatment. Clients discharged from (rather than maintained on) methadone displayed positive changes less often than any other group in treatment, except with regard to disability outcomes. In general, employment outcomes were least consistent across studies and appeared to be heavily affected by the performance of the local economy, as measured by the official unemployment rate.

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NATIONAL MULTISITE TREATMENT OUTCOME RESEARCH; THE DRUG ABUSE TREATMENT OUTCOME STUDY

R. L. Hubbard

National Development and Research Institutes

The Drug Abuse Treatment Outcome Study (DATOS) is the third in a series of treatment outcome research studies sponsored by the National Institute on Drug Abuse (NIDA). These studies were designed to develop information on clinical practice and to address scientific issues in treatment in community based settings. The studies used clinical epidemiological designs and methods with large samples of programs and patients. The studies were designed to describe treatment and clients, assess changes in behaviors and identify factors related to these changes.

Interviews were scheduled at admission to treatment and at key points during and after treatment to generate longitudinal data on the course of treatment and resultant periods of recovery and relapse. Each study addressed major core issues building on the knowledge and methods developed in the preceding research. The body of knowledge that has been accumulated and the advances in methods has provided a foundation for our understanding of how treatment works in community based settings. This understanding has helped guide funding decisions by policy makers and has suggested the need for clinical practice advances.

The series of studies was initiated with the Drug Abuse Reporting Program research in 1969-1973. That study conducted by Texas Christian University involved 44,000 patients from 139 methadone, outpatient drug free, long-term residential and detoxification programs across 35 cities. The programs reported data on clients. Follow-ups were conducted by the National Opinion Research Center (NORC) covering periods 1, 3, 6 and 12 years after treatment. The second study was the Treatment Outcome Prospective Study (TOPS) undertaken by the Research Triangle Institute with treatment admissions in the years 1979-1981. A total of 11,000 patients from 37 outpatient drug free, long-term residential and methadone programs in 10 cities were interviewed by trained study staff. Follow-ups were conducted three, twelve, twenty-four months and 3-5 years after termination from treatment. The Drug Abuse Treatment Outcome Study (DATOS) included 10,000 patients entering 96 short-term inpatient, long-term residential, outpatient drug free and methadone programs in 11 cities during the years 1991-1993. DATOS incorporated a much more extensive clinical assessment than the previous two studies. Follow-up interviews were conducted one and five years after discharge from treatment. The analysis of the data from DATOS has been coordinated under a cooperative agreement involving the National Development and Research Institutes, the Texas Christian University, the University of California at Los Angeles and the National Institute on Drug Abuse.

The DATOS research has reconfirmed the key findings first developed in the DARP research and then replicated in the TOPS data. DATOS has provided the third replication of the finding that clients reduce drug use during and after treatment and reduce their involvement in criminal activity. This finding was critical given the evolution of drug use patterns among patients from mainly opioids in DARP, to mixed patterns of multiples drugs in TOPS to mainly cocaine use in DATOS. DATOS also substantiated the finding of the 90-day retention threshold first identified in the DARP research and replicated in the TOPS data. The more detailed clinical assessment in DATOS also enabled the development of more extensive typologies of clients and measurement of severity. The use of similar measures of treatment and services first implemented in DARP and expanded in TOPS across the three studies provided the foundation for analysis of the influences of system level changes. In DATOS, major enhancements in the core therapies of programs, especially levels of methadone dosage and 12 step self-help groups, were found. These enhancements were unfortunately accompanied by major erosion in the nature and extent of comprehensive services for related mental health, family and employment problems. This erosion appeared in account for the lack of replication of the retention threshold effect on criminal activity and employment previously found in DARP and TOPS. The work in the DATOS cooperative has also led to advances in the modeling of treatment process, the application of addiction and treatment career concepts, and the incorporation of health services research perspective especially in the application of benefit cost models. The knowledge generated from the continuing, cooperative analysis of the extensive DARP, TOPS and DATOS databases serves as a model of community based research.

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DATOS Research Center (Robert L. Hubbard, Principal Investigator at NDRI) and two Collaborating DATOS Research Centers (M. Douglas Anglin, Principal Investigator at UCLA, and D. Dwayne Simpson, Principal Investigator at TCU) to conduct treatment evaluation studies in association with NIDA (Bennett W. Fletcher, Principal Investigator at NIDA). The interpretations and conclusions contained in this presentation do not necessarily represent the position of NIDA of the Department of Health and Human Services. We appreciate the time and help of the patients and program staff who participated in this research. Much of the information presented was developed for presentation at the TCU Forum: National Evaluations on Substance Abuse Treatment (May 9-10, 2000, Ft. Worth, Texas).

ANNUAL and PROGRESS REPORTS

University of Maryland and NIDDK/NIH

University of Mississippi, Louisiana State University and University of Michigan

University of Michigan

Medical College of Virginia of Virginia Commonwealth University

BIOLOGICAL EVALUATION OF COMPOUNDS FOR THEIR PHYSICAL DEPENDENCE POTENTIAL AND ABUSE LIABILITY. XXIV. DRUG EVALUATION COMMITTEE OF THE COLLEGE ON PROBLEMS OF DRUG DEPENDENCE (2000)

A. Coop (Biological Coordinator, DEC, CPDD), and A. E. Jacobson

Department of Pharmaceutical Sciences, University of Maryland School of Pharmacy, Baltimore, MD, and Laboratory of Medicinal Chemistry, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD

THE DRUG EVALUATION COMMITTEE (DEC) AND ITS MEMBERSHIP

Dr. A. Coop replaced Dr. A. E. Jacobson, the Biological Coordinator of DEC, CPDD, from 1976 to 2000. Dr. Coop is the fourth DEC Biological Coordinator (the initial two were Drs. N. Eddy and E. L. May). The other members of DEC remained unchanged this year; they are in DEC's two analgesic testing groups, at Virginia Commonwealth University (VCU, Drs. L. Harris, M. Aceto, E. Bowman, P. Beardsley) and the University of Michigan (UM, J. Woods (DEC Chair), J. Traynor), and three stimulant/depressant testing groups, at the University of Mississippi (UMs, W. Woolverton), Louisiana State University (LSU, C. France), and UM (G. Winger, J. Woods). Drs. T. Cicero and A. E. Jacobson act as emeritus members. The DEC reports to the CPDD's Liaison Committee for Drug Testing and Evaluation (A. Young, Chair). Members of both the CPDD Committee, and the Industry Relations Committee (R. Mansbach, Chair), as well as NIDA, attend DEC's meeting held during the Annual Scientific Meeting of the CPDD. One or two other DEC meetings are held during the year to discuss the work which has been accomplished, and future plans. Separate meetings are held at VCU quarterly with the members of the VCU Analgesic Testing Group, as well as Drs. E. L. May and E. Bowman, the DEC Biological Coordinator, and a NIDA representative, to discuss the results obtained from the VCU testing and research program.

Data were released for publication this year on 34 different compounds evaluated by DEC's Analgesic Testing Program. Of these, 33 compounds were evaluated at VCU (antinociceptive assays in mice - tail flick, hot plate, and phenylquinone, and the tail-flick antagonist assay, as well as substitution for morphine and precipitated withdrawal assays in rhesus monkeys), and 26 at UM (binding affinity to the μ, δ, and κ opioid receptors, and monkey self-administration) came from disparate sources: 65% from universities (56% from US universities and 9% from foreign universities); 21% of the compounds, more than usual, came from the pharmaceutical industry, and most of them (18%) were from US industry. Many of the remaining compounds (12%) came from governmental sources. A comparatively large number of compounds were released for publication this year (5 drugs) by the groups in the Stimulant/Depressant Testing Program. One of the compounds was examined to obtain data which the World Health Organization requested.

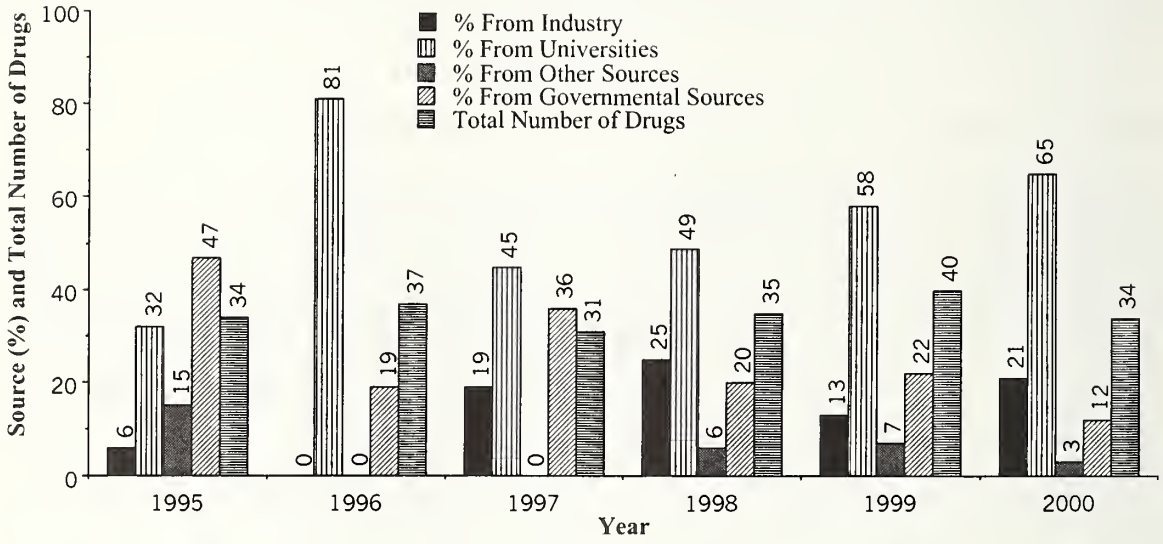
Two joint publications based on the data gathered under DEC auspices from MCV, UM and NIH, are in review or preparation (May *et. al.*, 2000a; May *et. al.*, 2000b).

EXPERIMENTAL OBSERVATIONS

The names of the compounds that were released for publication this year are listed in Table 1, and their molecular structures and a summary of their *in vivo* and *in vitro* data are in Tables 2 to 8. As in previous years (Jacobson, 2000), the examined compounds are classified according to their molecular structure, 4,5-epoxymorphinans in Table 2, morphinans in Table 3, and the 6,7-benzomorphans in Tables 4 and 5. Miscellaneous compounds (those which do not fall into the usual opioid classes) are listed in Tables 6 and 7, and the compounds evaluated by the Stimulant/Depressant testing groups are shown in Table 8. The more interesting compounds evaluated during the year are discussed below. For compounds that have been previously evaluated, the new data are discussed in relation to the published data.

STATISTICS

FIG. 1. DEC ANALGESIC PROGRAM: PERCENT, TOTAL NUMBER, AND SOURCE OF EXAMINED DRUGS (1995-2000)



NIH 10497 possesses an unusual *N*-1*R*-1-cyclopropylethyl substituent, similar to the μ-antagonist conferring *N*-cyclopropylmethyl. Previous reports (1989) showed that NIH 10497 is not active as a morphine antagonist in the mouse, and indeed can completely substitute for morphine in monkeys, indicating a μ-agonist profile. Side-effects seen in the monkeys (e.g. salivation) also suggested kappa agonist activity. Table 1 shows that NIH 10947 possesses high affinity at both μ and κ receptors, with somewhat lower affinity at δ receptors - thus the μ-agonist/κ-agonist activity seen in the monkey is consistent with the binding data. In contrast, GTPγS functional data show that NIH 10497 has low mu efficacy, which is not consistent with the *in vivo* substitution data. This is, however, consistent with the fact that NIH 10497 appears to be relatively free of μ-opioid dependence liability in the rat.

NIH 10924 (naltriben) is generally regarded as a δ_2-subtype selective antagonist, but we have reported that the pharmacology of natriben is complicated (Jacobson, 2000). Previous studies show that NIH 10924 is only moderately δ-opioid preferring (34- and 48-fold selective for δ- over μ- and κ-receptors, respectively). Indeed, potent morphine antagonism was seen in the mouse. Interestingly, NIH 10924 reversed DPDPE induced antinociception, even though DPDPE is considered a δ_1-subtype selective agonist. Lethal convulsions were also noted at high doses; these convulsions were not blocked by the δ-antagonist naltrindole, suggesting that they are not delta-receptor-mediated. Weak antinociception was reported in PPQ, but is probably not opioid receptor-mediated. As Table 2 shows, it is not reversed by nor-BNI or β-FNA. Thus, care must be taken when using naltriben as a δ_2-selective antagonist *in vivo*.

NIH 10968 in Table 2, 14-methoxymetopon, was found to be a potent and fairly selective μ-agonist. It was about fifty times more potent than morphine in the monkey single-dose-suppression assay, and had about that potency in antinociceptive assays, as well. This potency is somewhat less than noted in the literature as determined from an acetic acid writhing antinociceptive assay (Schmidhammer *et al.*, 1990). The 14-methoxy substituent appears to greatly enhance potency; metopon was previously found to be about three times more potent than morphine (Deneau and Seevers, 1955). **NIH 10998** (heterocodeine), in Table 2, was found to be a typical μ-agonist, more potent than, but otherwise quite similar to morphine. The increased C-ring hydrophobicity caused by blocking morphine's 6-hydroxy group apparently enhanced potency in a similar fashion to that seen for 6-acetylmorphine. Heterocodeine was initially examined in 1932 only in the hot plate assay (as NIH 00111), and it was found at that time to have an ED_{50} = 0.35 (0.31-0.39), when morphine's ED_{50} was about 2 mg/kg. NIH 00111 was seen to be a

little more potent than NIH 10998, which was found to have an ED_{50} = 0.51 (0.3-0.87) in the hot plate assay (morphine = 0.85 (0.39-1.86)), but the difference is not extreme, especially considering the 70 year time span.

The unusual endoethenomorphinan in Table 3 (**NIH 10931**) was a very potent long-acting antinociceptive. Interestingly, this compound, which was up to 2000 times more potent than morphine in the monkey single-dose-suppression assay, had opioid antagonist actions following the disappearance of its agonist activity, and this antagonist effect lasted for more than 168 hours. **NIH 10984**, Table 3, possesses a phenyl group fixed in a similar orientation to that in NIH 10931, and is similarly very active as a μ-opioid agonist. Unlike NIH 10931, NIH 10984 exhibited only agonist activity, and was not selective for a subtype of opioid receptor. Morphinan **NIH 10965** (Table 3), with unusual 4-benzyl ether, has been evaluated only in binding assays. It demonstrates relatively high μ-opioid affinity for a simple 3-methyl ether-substituted morphinan, indicating that a 4-benzyl ether is not detrimental to μ-affinity. The poor activity of NIH 10965 in the GTPγS functional assay at mu receptors, suggests that this compound will have mu antagonist properties.

An *N*-pent-4-ynylnormetazocine, **NIH 10972** in Table 4, was notable in that it was nonselective and not particularly potent as a μ-agonist. It antagonized morphine in the mouse (tail-flick), yet, surprisingly, substituted for morphine in the monkey (single-dose-suppression). In contrast, *N*-but-3-ynylnormetazocine, **NIH 10974**, was a potent agonist with good affinity for κ-opioid receptors (1.4 nM) and fair affinity for the remaining subtypes. NIH 10974 was inactive as a morphine antagonist in the mouse, and, as expected, substituted for morphine in the monkey (SDS). The *N*-methoxyethyl analogue in Table 4 (**NIH 10980**) was a potent agonist with high affinity for μ- and κ-receptors, and good affinity for δ-receptors, as well. The agonist activity in the mouse tail-flick was reversed by β-FNA, but not nor-BNI nor naltrindole, demonstrating μ-agonism. Thus, the fact that it did not substitute for morphine in the monkey (single-dose-suppression) is unusual. The change in activity, from antagonist to agonist, from relatively poor potency to high antinociceptive activity by modification of the N-substituent in opioids is not well understood even for compounds that mainly interact with the μ-opioid receptor, and is certainly less understood for those that interact with the other opioid receptors. These compounds are among those discussed in an article by DEC-associated authors (May *et al.*, 2000a). The corresponding (+)-isomers (which possess the unnatural opioid stereochemistry) are shown in Table 5. Most have very low affinity for opioid receptors, and the minor effects *in vivo* are probably non-opioid in nature.

Ketocyclazocine (**NIH 10964** in Table 5) was examined and found to be have potent antinociceptive activity. It was the prototypic κ-agonist (Iwamoto and Martin, 1981; Martin *et al.*, 1976),. In the present study, β-FNA, but not nor-BNI (a κ-antagonist) or naltrindole (a δ-antagonist), antagonized its agonist activity in the mouse tail flick assay, indicating that the drug acts as a the μ-opioid receptor agonist in that antinociceptive assay, and not as κ agonist. In fact, *in vitro* studies showed that NIH 10964 was nonselective and had almost equally high affinity to all three opioid receptors (*Ki* at μ = 6 nM, δ = 7 nM, and κ = 4 nM). Ketocyclazocine was examined previously as NIH 8847 in 1972 (hot plate ED_{50} = 0.4; it neither substituted for morphine nor precipitated withdrawal in monkey substitution studies), and as NIH 10346 in 1984 (hot plate ED_{50} = 0.65; partial suppression of abstinence observed in monkeys). Thus, similar hot plate assay results were observed over a span of 30 years. It was most recently found to be six times more potent than morphine, compared with three times more potent in 1972, and two times more potent in 1984. The results from monkey single dose suppression studies were only a little different. No substitution was found in the earliest study, and partial substitution was found both in 1984 and most recently. In this most recent study, the compound was found to precipitate withdrawal in the monkey. Its antagonist activity was found to be about 0.25 x naloxone. Since ketocyclazocine was inactive in the tail flick vs. morphine assay in mice, an assay for μ-antagonists, NIH 10964 could be acting as a κ-receptor antagonist in the monkey. In contrast to these data, GTPγS assays indicated that it has good potency and efficacy as a κ-agonist. It possessed much lower potency and efficacy at μ and δ receptors. **NIH 10993** (Table 5) is a racemic benzomorphan with an unusual acetamide N-substituent. This substituent removes almost all opioid activity; only weak binding to κ- and μ-receptors (304 and 677 nM, respectively) remains.

The notorious γ-hydroxybutyrate (GHB, **NIH 10947**) has unique properties as shown in Table 6. NIH 10947 has little opioid-like activity alone, but acts synergistically with morphine in PPQ. In addition, when NIH 10947 was given with morphine to morphine-tolerant mice, antinociception was partially restored. These data suggest potential therapeutic uses for GHB in the treatment of pain in morphine-tolerant patients, and potential safety issues for opioid abusers if they also administer GHB. Tramadol (**NIH 10969**, Table 6) and its symmetrical relative, **NIH 10970**, were examined and found to have little, if any, opioid-like activity. Tramadol is said to possess a μ-affinity

of about 2 μM (c.f. 3 μM Table 6) and to have codeine-like potency in man; its analgesic effects are said to be produced through both opioid and non-opioid mechanisms (Raffa *et al.*, 1992). The poor activity in the mouse, shown in Table 6, may be due to species differences, but it is in accord with our determined binding affinity.

NIH 10908 Sameridine (Table 6), which has been reported to possess local anaesthetic and analgesic effects, was found to be toxic in mice and to have relatively weak antinociceptive activity, probably mediated through μ-receptors (hot plate activity was antagonized by naloxone, AD_{50} = 0.07; completely substituted for morphine in the monkey). Carisoprodol (**NIH 10966**) (Table 6) was previously examined by the Stimulant/Depressant group (CPDD 0054). As expected, it had no opioid-like activity *in vivo* or *in vitro*. Further studies on NIH 10966 by the stimulant group are reported below. The ketobemidones (**NIH 11001** and **NIH 11002**) (Table 6) represent further examples of binding data that do not correlate with *in vivo* animal data, and underscore the importance for functional assays. NIH 11001 possesses about the same affinity for μ-receptors as, for example, heterocodeine (NIH 10998) (33 vs. 21 nM), yet NIH 11001 is completely inactive *in vivo*, both as an agonist and as an antagonist. NIH 11002 possesses a slightly lower affinity at μ-receptors (118 nM), and is also completely inactive *in vivo*.

The two enantiomers of norisonicotine (**NIH 10975** and **NIH 10976**) (Table 7) are under study for the treatment of cognitive dysfunction in conditions such as Alzheimer's disease (Levin *et al.*, 1999). Table 7 shows that they have no opioid actions. **NIH 10977** is a coumarin-based cyclic prodrug of the peptidic δ-opioid agonist DADLE. As shown in Table 7, peripheral administration of NIH 10977 gave rise to only slight antinociceptive effects in PPQ. The effect was not reversed by naltrindole, indicating a non-δ-opioid mediated effect. It appears that NIH 10977 does not enter the CNS. **NIH 10991** is currently receiving a great deal of attention as a potentially potent analgesic agent that acts through nicotinic rather than opioid receptors. Table 7 shows that NIH 10991 is indeed a potent antinociceptive agent, yet unlike previous reports ((Bannon *et al.*, 1998), the nicotinic antagonist mecamylamine did not reverse the antinociceptive effects. When combined with the toxic effects seen with NIH 10991, it is obvious that this compound requires further study to fully understand its pharmacology. **NIH 11008, NIH 11009, and NIH 11010** have been studied by both the analgesic and stimulant/depressant groups. (Table 8 gives their CPDD numbers). These compounds are *S*-(+), *R*-(-), and racemic mecamylamine, respectively. All three gave weak antinociception in PPQ (interestingly, the racemic NIH 11010 was the most potent) which was not reversed by naloxone or mecamylamine, demonstrating that the antinociception is neither opioid receptor mediated nor a nicotinic agonist effect. Indeed, all three compounds effectively antagonized the antinociceptive (tail-flick) effects of nicotine. The toxicity of these ligands (at 10 and 30 mg/kg) should be noted. **CPDD 0057** (NIH 11008) and **CPDD 0058** (NIH 11009) in the stimulant program showed no reinforcing effects in methohexital trained monkeys.

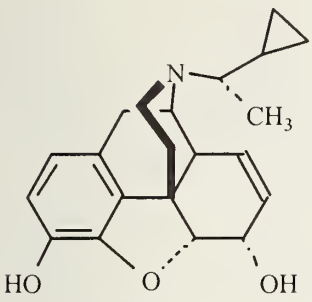
CPDD 0054 (NIH 10966) (Table 6) has been previously reported to possess no pentobarbital-like discriminative effects when administered i.g., however when administered i.v. full discrimination for pentobarbital was observed. CPDD 0054 may therefore have pentobarbital-like subjective effects in humans. **CPDD 0055** (Table 8) is a constituent in certain asthma medications; it displayed no stimulant effects. **CPDD 0056** (Table 8), a sulfur containing derivative of amphetamine, was inactive in the cocaine dependent monkey, and was not discriminated for the benzodiazepine midazolam at doses up to 3.2 mg/kg. Larger doses (10 mg/kg) of CPDD 0056 were lethal in the monkey. These data were generated at the request of the World Health Organization. Complete details on these drugs can be found in the Stimulant/Depressant Annual Report (France *et al.*, in press).

TABLE 1. EVALUATED COMPOUNDS

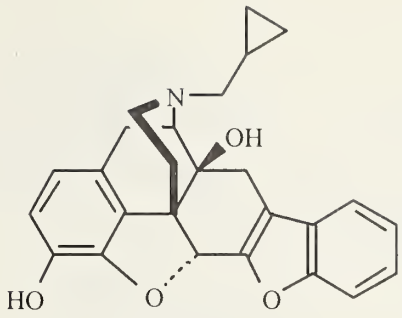
NIH#	COMPOUND NAME	TABLE #-Evaluator
10497	N-[(1*R*-1-Cyclopropylethyl]-N-normorphine hydrochloride	2-VCU
10908	Sameridine hydrochloride	6-VCU/UM
10924	Naltriben (NTB) methanesulfonate	2- VCU
10931	N-Methyl[5β,7β,3',5']pyrrolidino-2'-[*S*]-phenyl, 7α-methyl, 3-hydroxy, 6-methoxy-6,14-endoethenomorphinan dihydrochloride	3- VCU
10947	γ-Hydroxybutyric Acid, sodium salt	6-VCU/UM
10963	(±)-N-(But-3-ynyl)-N-normetazocine	4-VCU/UM
10964	(±)-8-Ketocyclazocine (also examined as NIH 8847 and 10346)	5-VCU/UM
10965	4-Benzyloxy-17-cyclopropylmethyl-14-hydroxy-17-nordihydrothebainone	3-UM
10966	Carisoprodol (also examined as CPDD 0054)	6-VCU/UM
10968	8-(Ethylmethylamino)-5,6,7,8-tetrahydroisoquinoline oxalate	2-VCU/UM
10969	Tramadol hydrochloride	6-VCU/UM
10970	2,6-Bis[(dimethylamino)methyl]-1-(3-methoxyphenyl)cyclohexanol dihydrochloride	6-VCU/UM
10971	(+)-(1*S*,5*S*,9*S*)-5,9-dimethyl-2'-hydroxy-2-(pent-4-ynyl)-6,7-benzomorphan	5- VCU/UM
10972	(-)-(1*R*,5*R*,9*R*)-5,9-dimethyl-2'-hydroxy-2-(pent-4-ynyl)-6,7-benzomorphan	4- VCU/UM
10973	(+)-(1*S*,5*S*,9*S*)-2-(But-3-ynyl)-5,9-dimethyl-2'-hydroxy-6,7-benzomorphan .HCl	5-VCU/UM
10974	(-)-(1*R*,5*R*,9*R*)-2-(But-3-ynyl)- 5,9-dimethyl-2'-hydroxy-6,7-benzomorphan .HCl	4-VCU/UM
10975	(-)-N-Norisonicotine .di-l-tartrate	7-VCU/UM
10976	(+)-N-Norisonicotine .di-d-tartrate	7-VCU/UM
10977	Coumarin-based cyclic prodrug of DADLE	7-VCU/UM
10980	(-)-(1*R*,5*R*,9*R*)-5,9-Dimethyl-2'-hydroxy-2-(2-methoxyethyl)-6,7-benzomorphan.HCl	4- VCU/UM
10981	(+)-(1*S*,5*S*,9*S*)-5,9-Dimethyl-2'-hydroxy-2-(2-methoxyethyl)-6,7-benzomorphan.HCl	5-VCU/UM
10982	(+)-(1*S*,5*S*,9*S*)-5,9-Dimethyl-2'-hydroxy-2-(3-hydroxypropyl)-6,7-benzomorphan.HCl	5-VCU/UM
10983	(-)-(1*R*,5*R*,9*R*)-5,9-Dimethyl-2'-hydroxy-2-(3-hydroxypropyl)-6,7-benzomorphan.HCl	4- VCU/UM
10984	E-3-Methoxy-4-hydroxy-5,14-ethano-18-(1-methyl)benzylidene-6-oxo-N-methylmorphinan	3-VCU/UM
10991	(*R*)-5-(2-Azetidinylmethoxy)-2-chloropyridine .*p*-toluenesulfonate	7-VCU/UM
10993	(-)-(1*R*,5*R*,9*R*)-2-Acetamido-5,9-dimethyl-2'-hydroxy-6,7-benzomorphan	5-VCU/UM
10998	Heterocodeine hydrochloride	2-UM
10999	(-)-(1*R*,5*R*,9*R*)-5,9-Dimethyl-2'-hydroxy-2-(3-trifluoromethylbenzyl)-6,7-benzomorphan hydrochloride	4-VCU/UM

11000	(+)-(1*S*,5*S*,9*S*)-5,9-Dimethyl-2'-hydroxy-2-(3-trifluoromethylbenzyl)-6,7-benzomorphan hydrochloride	5-VCU/UM
11001	4-(3-Hydroxyphenyl)-4-(1-oxopropyl)-1-(4-trifluoromethylbenzyl)piperidine .HCl	6-VCU/UM
11002	4-(3-Hydroxyphenyl)-4-(1-oxopropyl)-1-(3-trifluoromethylbenzyl)piperidine .HCl	6-VCU/UM
11008	*S*-(+)-Mecamylamine hydrochloride (see CPDD 0057)	7-VCU/UM
11009	*R*-(-)-Mecamylamine hydrochloride (see CPDD 0058)	7-VCU/UM
11010	(±)Mecamylamine hydrochloride (see CPDD 0059)	7-VCU/UM
CPDD 0055	(-)-Phenylephrine hydrochloride	8-S/D Group
CPDD 0056	4-Methylthioamphetamine hydrochloride	8-S/D Group
CPDD 0057	*S*-(+)-Mecamylamine hydrochloride (see NIH 11008)	8-S/D Group
CPDD 0058	*R*-(-)-Mecamylamine hydrochloride (see NIH 11009)	8-S/D Group
CPDD 0059	(±)Mecamylamine hydrochloride (see NIH 11010)	8-S/D Group

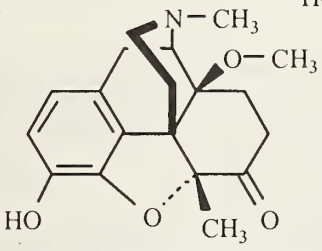
TABLE 2. 4,5-EPOXYMORPHINANS



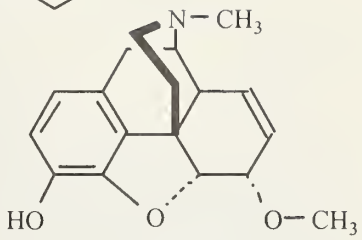
NIH 10497



NIH 10924



NIH 10968



NIH 10998

ANTINOCICEPTIVE/ANTAGONIST ASSAYS IN VITRO MONKEY (MOUSE ED50/AD50, s.c., mg/kg)

NIH #	Hot Plate	Phenylquinone	Tail Flick	Tail Flick Antagonist	Binding Affinity, nM	Substitution-for-Morphine (s.c., mg/kg)
10497	-	0.03[a]	2.0[a]	Inactive[a]	μ=0.1, δ=29, κ=1.3[b]	Complete substitution[a,c]
10924	Inactive[d]	4.2[d,e]	Inactive[d,e]	0.99[c]	μ=12.4, δ=0.36, κ=17.5[c]	-
10968	0.03	0.009	0.03	Inactive	μ=0.03, δ=41, κ=304	Complete substitution (50 x morphine)
10998	0.51	0.04	0.21[f]	Inactive	μ=21, δ=251, κ=271	Complete substitution (2 x morphine)

a) Previously reported 1989.

b) GTPγS assay: mu EC_{50} = 2191 ± 773 nM (18.7 ± 5.8% stimulation); delta EC_{50} = 72.2 ± 21.0 nM (11.7 ± 3.3% stimulation; kappa EC_{50} = 18.3 ± 4.1 nM (78.4 ± 3.8% stimulation).

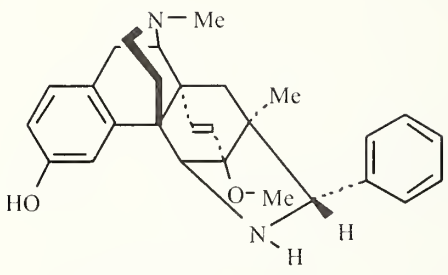
c) Monkey self-administration: maintained rates between saline and codeine; monkey drug discrimination: codeine like; thermal analgesia: μ + κ, more effective @ 50 than 55 °C; rat primary physical dependence: relatively free of μ-opioid dependence liability; naloxone AD50 (tail flick): 2.98; vas deferens[a]: κ-profile; rat brain homogenate binding[a]: 2.1 nM.

d) Previously reported 1998

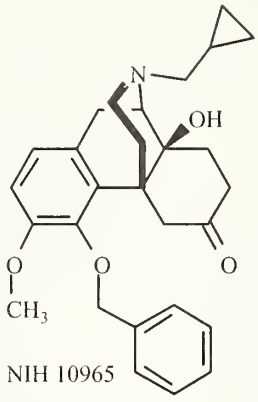
e) Convulsions, lethal @ 30 mg/kg. Naltrindole pretreatment did not abolish lethal effects[c]; 10924 vs. DPDPE (i.c.v., tail flick) AD_{50} = 3.2,[c] naltrindole (s.c., tail flick) vs 10924 = inactive[c]. μ- & δ-antagonist, agonist in PPQ[c] ; 10924 vs ED80 DPDPE (i.c.v., PPQ) AD50 = 3.2 (previously reported 1999); nor-BNI (s.c.) and β-FNA (i.c.v.) vs 10924 in PPQ: inactive.

f) Naloxone vs ED_{80} of 10998: AD_{50} = 0.04; opioid subtype: β-FNA (i.c.v.) vs ED_{80}: AD_{50} = 0.06 μg/brain; nor-BNI and naltrindole vs ED_{80}: inactive.

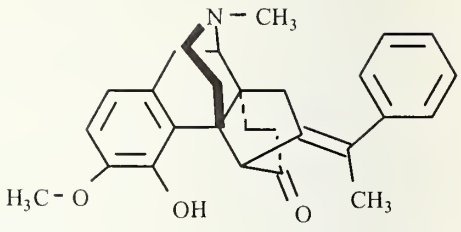
TABLE 3. MORPHINANS



NIH 10931



NIH 10965



NIH 10984

ANTINOCICEPTIVE/ANTAGONIST ASSAYS (MOUSE ED50/AD50, s.c., mg/kg) **IN VITRO** **MONKEY**

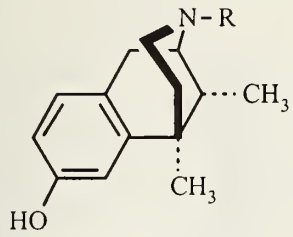
NIH #	Hot Plate	Phenylquinone	Tail Flick	Tail Flick Antagonist	Binding Affinity, nM	Substitution-for-Morphine (s.c., mg/kg)
10931	0.01	0.004	0.005[a]	Inactive	–	Complete suppression (potency 500-2000 x morphine)
10965	-	-	-	-	μ=10.4, δ= 6476, κ=202[b]	-
10984	0.1	0.012	0.05[c]	Inactive	μ=0.9, δ=6.7, κ=0.4	Complete suppression (potency 100 x morphine)

a) Naloxone AD50 = 0.02. Time course study (mice): Long duration, potent antinociceptive, followed by antagonist activity for > 168 hours. Drug naïve monkeys: μ- and κ-agonist, μ-antagonist, muscarinic effects on acute administration.

b) GTPγS assay: mu EC_{50} = 1254 ± 576 nM (21.4 ± 10.6% stimulation)

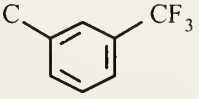
c) β-FNA (i.c.v., tail flick) vs ED_{80} 10984: AD_{50} = 1.2 (nor-BNI and naltrindole: inactive) - potent μ-agonist. No antagonism of morphine ED_{80} after 10984 pretreatment from 2 to 120 hours.

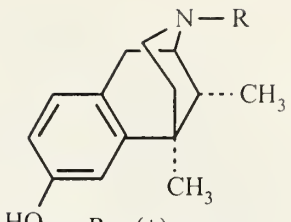
TABLE 4. 6,7-BENZOMORPHANS



(-)-(2*R*,5*R*,9*R*)

R =

10974: $CH_2CH_2C{\equiv}CH$
10972: $CH_2CH_2CH_2C{\equiv}CH$
10980: $CH_2CH_2OCH_3$
10983: $CH_2CH_2CH_2OH$
10999: H_2C  CF_3



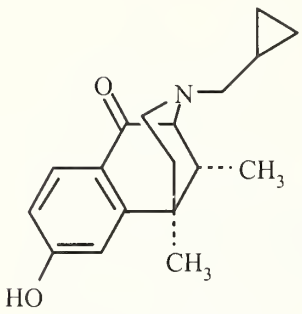
R = (±)-
10963: $CH_2CH_2C{\equiv}CH$

ANTINOCICEPTIVE/ANTAGONIST ASSAYS (MOUSE ED50/AD50, s.c., mg/kg) **IN VITRO** **MONKEY**

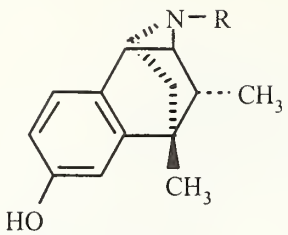
NIH #	Hot Plate	Phenylquinone	Tail Flick	Tail Flick Antagonist	Binding Affinity, nM	Substitution-for-Morphine (s.c., mg/kg)
10963	0.14	0.04	0.18[a]	Inactive	μ=0.8, δ=15, κ=3	Complete substitution (potency 6 x morphine)
10972	Inactive	11.8[b]	Inactive	2.0	μ=18, δ=137, κ=11	Complete substitution
10974	0.13	0.04[c]	0.05	Inactive	μ=4.6, δ=10, κ=1.4	Complete substitution
10980	0.15	0.01	0.07[d]	Inactive	μ=0.5, δ=9, κ=0.8	No sustitution[e]
10983	Inactive	11.6	Inactive	Inactive	μ=34, δ=124, κ=137	Exacerbated withdrawal. Weak agonist-antagonist.
10999	Inactive	Inactive	Inactive	Inactive	μ=314, δ=2904, κ=704	Partial substitution (brief)

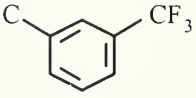
a) Nor-BNI or naltrindole vs ED_{80} 10963 are inactive; β-FNA vs ED_{80}: AD_{50} = 0.03.
b) β-FNA (i.c.v.) vs ED_{80} of NIH 10963: 43% maximum @10 μg/brain; nor-BNI or naltrindole: inactive; 10972 vs ED80 DPDPE (i.c.v., tail flick): AD50 = 0.05. Weak μ-agonist, μ-, κ-, δ-antagonist.
c) Naltrindole, nor-BNI, and β-FNA (s.c., s.c., i.c.v., respectively, tail flick) vs ED_{80} 10974: AD50: 0.29, 3.1, 0.64, respectively.
d) β-FNA (i.c.v., tail flick) vs ED_{80} 10980: AD50: 3.1, and nor-BNI or naltrindole: inactive.
e) Non-dose related attenuation of withdrawal. Incomplete substitution even at doses inducing overt μ- or κ-behavioral effects.

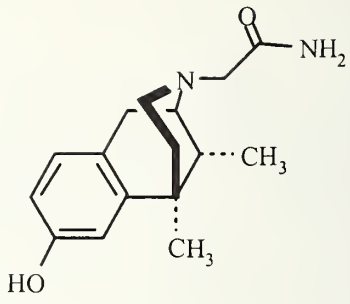
TABLE 5. 6,7-BENZOMORPHANS (CONTINUED)



NIH 10964 (±)



(+)-2*S*,5*S*,9*S*) R =
10973: $CH_2CH_2C{\equiv}CH$
10971: $CH_2CH_2CH_2C{\equiv}CH$
10981: $CH_2CH_2OCH_3$
10982: $CH_2CH_2CH_2OH$
11000: 



NIH 10993 (±)

ANTINOCICEPTIVE/ANTAGONIST ASSAYS IN VITRO MONKEY (MOUSE ED50/AD50, s.c., mg/kg)

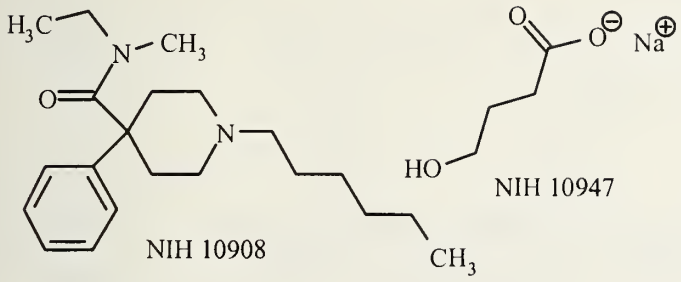
NIH #	Hot Plate	Phenylquinone	Tail Flick	Tail Flick Antagonist	Binding Affinity, nM	Substitution-for-Morphine (s.c., mg/kg)
10964	0.14	0.12	0.45[a]	Inactive	μ=6, δ=7, κ=4[b]	Partial suppression[c]
10971	Inactive	14.8	Inactive	Inactive	μ=3572, δ=>10000, κ=328	No substitution - exacerbates withdrawal
10973	Inactive	Inactive	Inactive	Inactive	μ=>10000, δ = >10000, κ=1726	Non-dose related attenuation of withdrawal
10981	Inactive	22.7	Inactive	Inactive	μ=778, δ=5712, κ=1158	Partial suppression
10982	Inactive	Inactive	Inactive	Inactive	μ=2102, δ=>10000, κ=915	Partial suppression. Non-dose related.
10993	Inactive	3.5	Inactive	Inactive	μ=677, δ=2005, κ=304	No substitution, no exacerbation of withdrawal.
11000	Inactive	Inactive	Inactive	Inactive	μ=598, δ=1644, κ=528	No substitution, no exacerbation of withdrawal

a) β-FNA (s.c.) vs. ED_{80} of NIH 10972: AD50 = 5.7; nor-BNI and naltrindole: inactive

b) GTPγS assay: mu EC_{50} = 273 ± 103 nM (19.1 ± 4.9% stimulation); delta EC_{50} = 122 ± 35 nM (45.9 ± 9.6% stimulation); kappa EC_{50} = 14.3 ± 1.6 nM (91.0 ± 4.3% stimulation).

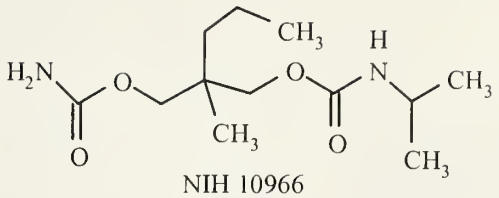
c) Precipitated withdrawal: antagonist, potency 0.25 x naloxone.

TABLE 6. MISCELLANEOUS

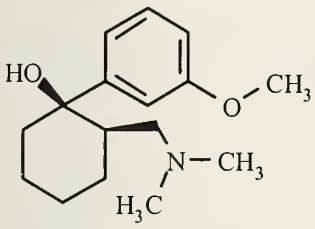


NIH 10908

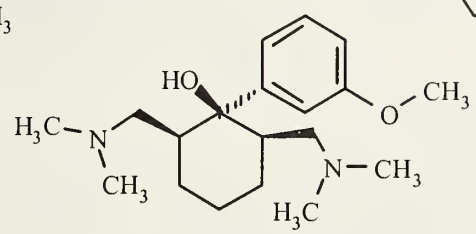
NIH 10947



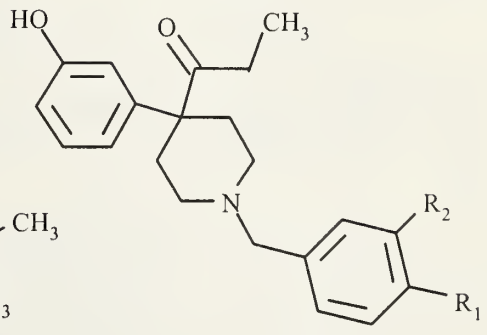
NIH 10966



NIH 10969



NIH 10970



	R_1	R_2
NIH 11001:	CF_3	H
NIH 11002:	H	CF_3

ANTINOCICEPTIVE/ANTAGONIST ASSAYS IN VITRO MONKEY
(MOUSE ED50/AD50, s.c., mg/kg)

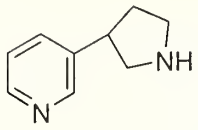
NIH #	Hot Plate	Phenylquinone	Tail Flick	Tail Flick Antagonist	Binding Affinity, nM	Substitution-for-Morphine (s.c., mg/kg)
10908	8.8[a]	2.5	7.4	Inactive	–	Complete substitution
10947	-	iv: 31[b]	i.v., s.c., p.o.: inactive	-	–	Inverse dose-response.
10966	Inactive	Inactive	Inactive	Inactive	μ, δ, κ =>10000	No effect.
10969	Inactive	6.1[c]	Inactive	Inactive	μ = 2995, δ, κ =>10000	-
10970	Inactive	Inactive	Inactive	Inactive	μ, δ, κ =>10000	-
11001	Inactive	Inactive	Inactive	Inactive	μ=33, δ=291, κ=118	No substitution
11002	Inactive	Inactive	Inactive	Inactive	μ=118, δ=316, κ=203	No substitution

a) Unusually toxic to mice. Naloxone AD_{50} (tail flick): 0.07.

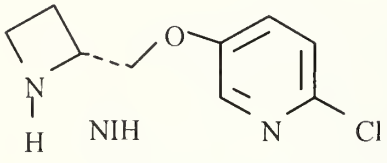
b) Co-administration (20-100 mg/kg, s.c.) with ED25 morphine: dose-related synergism. Morphine-tolerant mice: 10947 (GHB) + morphine partially restored antinociception (abolished by naloxone).

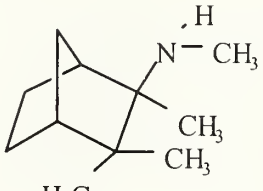
c) Naltrindole (s.c., PPQ) vs 10969 ED80: inactive.

TABLE 7. MISCELLANEOUS (CONTINUED



NIH 10975:(-)
NIH 10976:(+)





NIH 11008:*S*-(+)
NIH 11009:*R*-(-)
NIH 11010:

ANTINOCICEPTIVE/ANTAGONIST ASSAYS IN VITRO MONKEY (MOUSE ED50/AD50, s.c., mg/kg)

NIH #	Hot Plate	Phenylquinone	Tail Flick	Tail Flick Antagonist	Binding Affinity, nM	Substitution-for-Morphine (s.c., mg/kg)
10975	Inactive	Inactive	Inactive	Inactive	μ, δ, κ =>10000	Weak, non-dose related attenuation of withdrawal
10976	Inactive	Inactive	Inactive	Inactive	μ, δ, κ =>10000	No effect
10977	Inactive	32.5[a]	Inactive	Inactive	μ, δ, κ =>10000	-
10991	2.57	0.004[b]	Inactive	Inactive	-	-
11008[f]	-	11.4[c,d]	Inactive	-	-	-
11009[f]	-	9.5[c,e]	Inactive	-	-	-
11010[f]	-	4.2 [c,d]	Inactive	-	-	-

a) Naltrindole vs 10977 ED80 (PPQ): inactive. Tail flick (i.v. and s.c., 90 min pretreat): inactive.

b) Toxic, convulsions. β-FNA (i.c.v.), naltrindole (s.c.), or mecamylamine (s.c.) vs ED80 10991: <60%, and nor-BNI: AD50 = 11.3. Actions not nicotine-related.

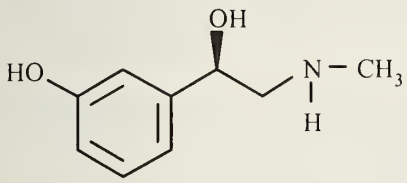
c) Neither mecamylamine nor naloxone (pretreatment) antagonized ED80.

d) 6/6 Mice died @ 30 mg/kg (iv).

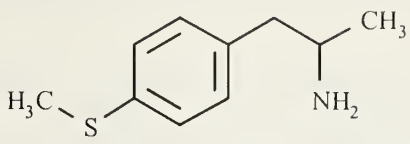
e) 4/6 Mice died @ 10 mg/kg (iv); immobile, tremors.

f) Special test: Effect vs. ED80 of nicotine in tail-flick: NIH 11008 AD50 = 0.03; NIH 11009 AD50 = 0.12; NIH 11010 AD50 = 0.36.

TABLE 8. EVALUATION OF STIMULANT/DEPRESSANT DRUGS



CPDD 0055



CPDD 0056

CPDD#	Discriminative Stimulus Effects in Monkeys. Comparison to Flumazenil & Midazolam (s.c.)	Monkey Self-Administration (iv)	Monkey Drug Discrimination (i.g.)
0054; NIH 10966[a]	No benzodiazepine discriminative stimulus effects[c]	-	Did not discriminate for amphetamine[c] Discriminated for pentobarbital i.v., (but not i.g.)[c]
0055	No benzodiazepine discriminative stimulus effects	No reinforcing effects in cocaine dependent monkey	No amphetamine discriminative effects at doses up to 10mg/kg
0056	Toxic, 10 mg/kg	No reinforcing effects in cocaine dependent monkey	-
0057; NIH 11008[b]	-	No reinforcing effects in methohexital trained monkeys	-
0058; NIH 11009[b]	-	No reinforcing effects in methohexital trained monkeys	-
0059; NIH 11010[b]	-	-	-

a) See Table 6 for molecular structure.
b) See Table 7 for molecular structure.
c) Previously reported 2000.

NOTES FOR TABLES 2 - 8

Rounded numbers are used; precise values and details of the procedures are given in the VCU and UM reports (Aceto *et al.*, 2001; Woods *et al.*, 2001).

1) Antinociceptive reference data:
Morphine ED_{50} (confidence limits): Hot Plate = 0.8 (0.3-1.8); Phenylquinone = 0.23 (0.20-0.25); Tail-Flick = 5.8 (5.7-5.9)
Tail-Flick Antagonism vs. morphine (naltrexone AD_{50}= 0.007 (0.002-0.02); naloxone AD_{50}= 0.035 (0.01-0.093)).
2) *In Vitro* - Subtype selective binding affinity using monkey brain cortex membranes. Selectivity for μ, δ, and κ-opioid receptors determined with [^{3}H]-DAMGO, [^{3}H]-*p*-Cl-DPDPE and [^{3}H]-U69,593, respectively. Affinities of labeled ligands: [^{3}H]DAMGO K_i = 0.57 nM, [^{3}H]*p*-Cl-DPDPE K_i = 1.2 nM, [^{3}H] U69,593 K_i = 0.95 nM. With C6 glioma cells, morphine K_i = 1.7 nM, DPDPE K_i = 8 nM.

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PROGRESS REPORT FROM THE TESTING PROGRAM FOR STIMULANT AND DEPRESSANT DRUGS (2000)

K.G. Anderson, R. Ranaldi, W.L. Woolverton, C.P. France, L.R. Gerak, and G. Winger

University of Mississippi Medical Center, Jackson, MS; Louisiana State University Health Sciences Center, New Orleans, LA; University of Michigan, Ann Arbor, MI

INTRODUCTION

The research group involved in the evaluation of stimulant and depressant compounds has been in existence for approximately 15 years. The group includes laboratories at Louisiana State University Health Sciences Center (Gerak, France), University of Mississippi Medical Center (Anderson, Ranaldi, Woolverton), and the University of Michigan (Winger, Woods) and is part of the Drug Evaluation Committee (Dr. J. Woods, Chair) of the College on Problems of Drug Dependence (CPDD) which is supported by both CPDD and the National Institute on Drug Abuse (NIDA). The participating laboratory from Louisiana State University was relocated to the University of Texas Health Science Center at San Antonio. One of the purposes of the group is to evaluate new compounds, generally classified as either stimulants or depressants, for their abuse liability and physical dependence potential. Compounds are received, coded and distributed by Drs. A. Jacobson at the National Institute of Diabetes, Digestive and Kidney Diseases (NIDDK), National Institutes of Health (NIH) and A. Coop at the University of Maryland, for blind testing in the various laboratories. They are evaluated for reinforcing effects in monkeys that previously self-administered cocaine or methohexital (UM), and for discriminative stimulus effects in pentobarbital-trained monkeys (UMMC), *d*-amphetamine-trained monkeys (UMMC), midazolam-trained monkeys (LSUHSC), and flumazenil-trained monkeys that receive diazepam daily (LSUHSC). This report includes the results of evaluation of CPDD-0055 through CPDD-0058 and an update of previously published work with CPDD-0054 (See NIDA Research Monograph 180, 1999). All studies were conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee, Louisiana State University Health Sciences Center New Orleans, University of Mississippi Medical Center, University of Michigan, and the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health.

METHODS

Reinforcing Effects in Rhesus Monkeys (UM)

Subjects

Subjects were rhesus monkeys (*Macaca mulatta*) experienced with self-administration of cocaine hydrochloride or sodium methohexital. Animals were surgically prepared with indwelling silicone rubber catheters using 10 mg/kg intramuscular (i.m.) ketamine and 2.0 mg/kg i.m. xylazine as anesthetics. Catheters were implanted in jugular (internal or external), femoral or brachial veins as necessary. Catheters passed subcutaneously (s.c.) to the mid-scapular region, exited the body and continued, through a hollow restraining arm, to the outside rear of the cage.

Apparatus

An earlier version of the restraint and catheter protection devices are described in detail by Deneau et al. (1969). Each monkey wore a teflon cloth jacket that attached to a flexible tubular restraining arm (Lomir Biomedical, Malone, NY) that carried the catheter to the back of the cage where it joined tubing passing through a roller infusion pump (Watson and Marlow Co., Model MHRK 55, Falmouth, UK).

Monkeys were individually housed in stainless steel cages, measuring 83.3 X 76.2 X 91.4 cm deep. A 15.4 cm square stimulus panel was located on the side of each cage, approximately 10 cm from the front and 19 cm from the bottom of the cage. Across the top of the stimulus panel, 1.5 cm apart, were three circles, 2.5 cm in diameter, covered with translucent plastic and capable of being illuminated from behind by 5-w colored bulbs. The two side lights could be illuminated red and the center light green. Below each of the two red stimulus lights was a response lever (Model 121-07; BRS-LVE, Beltsville, MD) capable of being operated by a force of 0.010 to 0.015 N. Experimental control was provided by an IBM PS/2 computer programmed with Med-PC (Med-Associates, Fairfield, VT) software and located in an adjoining room.

Procedure

Reinforcing effects of CPDD-0055 and CPDD-0056 were evaluated in a substitution self-administration procedure in monkeys who were experienced with intravenous (i.v.) self administration of cocaine. The subjects were given the opportunity to respond and receive cocaine or saline infusions through intravenously implanted catheters during two 130-minute sessions each day. Each session was divided into four components of 25 minutes or 20 injections, whichever came first. The components were separated from each other by a 10-minute blackout period, during which time all stimulus lights were extinguished, and lever responses had no programmed consequence. The duration of the i.v. infusion that served to reinforce behavior was different in each of the four components. This made four different doses of cocaine available to the monkeys at different times during sessions in which cocaine was the available reinforcer. At the beginning of each session, a red light was illuminated over one of two levers. When the light was illuminated, 30 responses on that lever [fixed-ratio (FR) 30] resulted in an intravenous infusion of drug or saline. Each infusion was followed by a 45-second timeout; during the infusion and the timeout, the red light was extinguished. There was a centrally located green light illuminated during the infusions. After each timeout, the red light was again turned on.

On approximately half of the baseline sessions, cocaine was used to maintain behavior; response-contingent saline was available during the other baseline sessions. The range of cocaine doses was 0.001-0.03 mg/kg/inj. Prior to substitution of CPDD-0055 or CPDD-0056, each monkey was required to show a clear and consistent dose-response curve with cocaine and consistently low rates of responding throughout the session when saline was substituted for cocaine. A range of doses of CPDD-0055 and CPDD-0056 was evaluated in the monkeys using this procedure.

The reinforcing effects of CPDD-0057 and CPDD-0058 were evaluated in three monkeys that were experienced with i.v. self-administration of sodium methohexital. The subjects were given the opportunity to respond and receive methohexital or saline infusions through intravenously implanted catheters during two 130-minute sessions each day. At the beginning of each session, a red light was illuminated over one of two levers. When the light was illuminated, 10 responses (FR 10) on that lever resulted in an i.v. infusion of 0.1 mg/kg/inj of methohexital or saline. Each infusion was followed by a 10-second timeout; during the infusion and the timeout, the red light was extinguished. There was a centrally located green light illuminated during the infusions. After each timeout, the red light was again turned on.

On approximately half of the baseline sessions, 0.1 mg/kg/inj sodium methohexital was available; response-contingent saline was available on the other baseline sessions. Prior to substitution of CPDD-0057 or CPDD-0058, each monkey was required to show consistent levels of behavior maintained by sodium methohexital that were markedly greater than those shown by saline. A range of doses of CPDD-0057 and CPDD-0058 was evaluated in the monkeys using this procedure.

Drugs

Doses of CPDD-0055 and CPDD-0056 were manipulated by varying the duration of the infusion. Because only four doses for a given compound could be evaluated in a single session, a larger dose range was studied by making different concentrations of the test drug available on different sessions. For example,

0.01-0.3 mg/kg/inj of CPDD-0055 or CPDD-0056 was evaluated in one session, and 0.03-1.0 mg/kg/inj CPDD-0055 or CPDD-0056 was evaluated in a session several days later.

Three doses of CPDD-0057 (0.01, 0.03, and 0.1 mg/kg/inj) were evaluated in the monkeys and each dose was tested twice. At the largest tested dose of 0.1 mg/kg/inj, one monkey showed behavioral changes after the session that indicated that behaviorally active doses had been used. Therefore, larger doses were not evaluated in any of the monkeys. Four doses of CPDD-0058 (0.01, 0.03, 0.1, and 0.3 mg/kg/inj) were evaluated and each dose was tested twice. At the largest tested dose of 0.3 mg/kg/inj, one monkey showed behavioral changes after the session that indicated that behaviorally active doses had been used. Therefore, larger doses were not evaluated in any of the monkeys. Cocaine hydrochloride and methohexital sodium were dissolved in sterile 0.9% saline and sterile water, respectively. All the CPDD test compounds were dissolved in sterile 0.9% saline.

Discriminative Stimulus Effects in Rhesus Monkeys (pentobarbital and *d*-amphetamine discriminations, UMMC)

Subjects

The subjects were eight adult rhesus monkeys weighing between 6.4 and 12.2 kg. Monkeys were housed individually in stainless steel cages in which water was available continuously. They were fed 150 to 200 grams of Teklad monkey chow after each session and were given a chewable vitamin tablet three times per week.

The monkeys had been trained previously to discriminate *d*-amphetamine (Ou3, 8405, and 8515) or pentobarbital (AQ63, Ef3, 8814 and 8902) from saline in a two-lever, discrete-trial shock avoidance procedure. Monkey M163 was trained to discriminate *d*-amphetamine from saline in a food-maintained procedure. All monkeys had received other test drugs prior to CPDD-0054, CPDD-0055, or CPDD-0056.

Apparatus

During experimental sessions animals were seated in primate restraint chairs and placed inside sound-attenuating cubicles. All chairs were fitted with shoes containing brass plates in the soles that permitted delivery of electric shock from a shock generator (SG 903 BRS/LVE, Laurel, MD). Chambers were equipped with two response levers (PRL-001, BRS/LVE, Laurel, MD) mounted on one wall. There were four white lights above each lever. Chambers were illuminated with ceiling-mounted 40-w incandescent house lights. Experimental events were programmed and recorded with an Apple Macintosh II computer that was located in a room adjacent to the experimental room.

Procedure

The training and test procedures have been reported in detail elsewhere (Woolverton et al., 1994). A monkey was placed in the restraint chair and either saline (1-2 ml) or the training drug was administered intragastrically (i.g.) via a nasogastric tube, followed by a 1.5 ml saline flush. Fifty-five minutes after infusion, the monkey was placed in the experimental chamber.

The session began with a 5-minute timeout that was followed by 30 trials. On each trial the house light and lever lights were illuminated and responding on the correct lever postponed scheduled shock and extinguished the lights. Incorrect responses reset the response requirement on the correct lever. The correct lever was determined by the pre-session infusion (drug or saline). If the response requirement (FR 5) was not satisfied on the correct lever within 10 seconds of the onset of the lights, shock (250-msec duration, 5-mA intensity) was delivered. If the response requirement was not satisfied within four additional seconds, a second shock was delivered and the trial ended. The session was terminated when two shocks were delivered during two consecutive trials or after 30 trials. Trials were separated by 30-second timeouts.

For the monkey with lever pressing maintained by a FR 5 schedule of food presentation (M163), the discrimination was between 1.7 mg/kg *d*-amphetamine and saline. Sessions ended after 20 minutes and there were no timeouts.

Training sessions were conducted five days a week according to the following schedule: SDDSS, DSSDD, where S denotes sessions preceded by saline and D denotes sessions preceded by drug. Discrimination training continued until at least 90% of the responses in the first trial were on the correct lever and subjects avoided shock on at least 90% of the trials (27/30) for seven out of eight consecutive sessions. In the food-maintained procedure, discrimination criteria were satisfied when 90% of responses (overall responding and responding prior to the delivery of the first food pellet) were emitted on the drug-appropriate lever for seven out of eight consecutive sessions. When subjects failed to satisfy criteria, the training sequence was conducted until the criteria were once again satisfied. Test sessions were identical to training sessions except that test drugs were administered and completing the response requirement on either lever avoided shock.

Drugs

A stock solution made from *d*-amphetamine sulfate (NIDA, Rockville, MD) was prepared by dissolving drug in sterile 0.9% saline in a concentration of 5.0 mg/ml. The training dose of *d*-amphetamine was 1.0 or 1.7 mg/kg, i.g. Pentobarbital was mixed daily by diluting Nembutal (Abbott Laboratories, N. Chicago, IL) with sterile 0.9% saline. The training dose of pentobarbital was 10 mg/kg, i.g. CPDD-0054 was dissolved in 1:1:1 DMSO:ethanol:0.9% saline immediately before administration and infused at a volume of 0.25 ml/kg. CPDD-0054 was tested up to a dose of 30.0 mg/kg, i.v., CPDD-0055 was tested up to a dose of 30.0 mg/kg, and CPDD-0056 was tested up to a dose of 3.0 mg/kg. CPDD-0055 and CPDD-0056 were evaluated via the i.g. and i.m. routes of administration and were dissolved in sterile 0.9% saline and given at a volume of 0.25 ml/kg (i.g.) and 1.0 ml/kg (i.m). Compounds administered via the i.g., i.m., and i.v. routes were given 60, 15, and 5 minutes prior to the start of the experimental session, respectively.

Discriminative Stimulus Effects in Rhesus Monkeys (flumazenil and midazolam discriminations, LSUHSC)

Subjects

The subjects were six rhesus monkeys weighing between 3.5 and 10.5 kg. Monkeys were housed individually in stainless steel cages in which water was continuously available and they received primate chow (Harlan Teklad, Madison, WI) daily as well as fresh fruit and peanuts daily.

Apparatus

Monkeys were seated in chairs that provided restraint at the neck. During experimental sessions, chairs were located in sound-attenuating, ventilated chambers that were equipped with several response levers, a food cup and an array of stimulus lights. Chairs were equipped with shoes containing brass electrodes, to which brief (250 msec) electric shock could be delivered from an a.c. shock generator located adjacent to the chambers.

Procedure

Flumazenil Discrimination. Monkeys consumed 5.6 mg/kg of diazepam in 55-60 ml of fruit punch 3 hrs prior to daily sessions in which they discriminated between s.c. injections of 0.32 mg/kg of flumazenil and vehicle while responding under a FR 5 schedule of food presentation (Gerak and France, 1999). Daily training sessions consisted of several discrete, 15-minute cycles. Each cycle comprised a 10-minute pretreatment period, during which the chamber was dark and lever presses had no programmed consequence, followed by a response period, during which the chamber was illuminated green and monkeys could receive a 300 mg banana-flavored food pellet by responding five times on the appropriate lever as

determined by the s.c. injection administered during the first minute of the 10-minute timeout (e.g., left lever after vehicle, right lever after flumazenil).

Test sessions were identical to training sessions except that various doses of flumazenil or a test compound (CPDD-0054, CPDD-0055) were administered during the first minute of each timeout and five consecutive responses on either lever resulted in food delivery.

Midazolam Discrimination. Monkeys discriminated between s.c. injections of 0.56 mg/kg of midazolam and vehicle while responding under a FR 5 schedule of stimulus-shock termination (Lelas et al., 1999). Daily sessions comprised multiple, 15-minute cycles. Each cycle comprised a 10-minute timeout, during which the chamber was dark and lever presses had no programmed consequence, followed by a response period, during which the chamber was illuminated red and monkeys could postpone scheduled shock for 30 seconds by responding five times on the appropriate lever as determined by the s.c. injection administered during the first minute of the 10-minute timeout (e.g., left lever after vehicle, right lever after midazolam). Failure to satisfy the response requirement within 10 seconds resulted in the delivery of a brief shock. The response period ended after 5 minutes or the delivery of four shocks, whichever occurred first. Responses on the injection-inappropriate lever reset the response requirement on the correct lever.

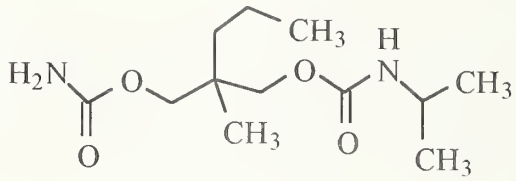
Test sessions were identical to training sessions except that various doses midazolam or a test compound (CPDD-0054, CPDD-0055, CPDD-0056) were administered during the first minute of the timeout and five consecutive responses on either lever postponed the shock schedule.

Drugs

Diazepam (Zenith Laboratories, Northvale, NJ) was suspended in 55-60 ml (depending on body weight) of fruit punch containing suspending Agent K to yield a dose of 5.6 mg/kg/daily drinking episode. Flumazenil (F. Hoffman LaRoche, LTD, Basel, Switzerland) was dissolved in a vehicle of 10% ethanol, 40% propylene glycol and 50% saline; midazolam hydrochloride (Roche Pharma, Inc., Manati PR) was purchased as a commercially-prepared solution. CPDD 0054 was dissolved in a vehicle comprising 25% ethanol, 20% emulphor and 55% saline and was studied up to a dose of 32.0 mg/kg, s.c. CPDD 0055 was dissolved in sterile water and was studied up to a dose of 32.0 mg/kg, s.c. CPDD 0056 was dissolved in sterile water and was studied up to a dose of 10.0 mg/kg, s.c., but not all monkeys received all doses (see below).

RESULTS

CPDD-0054
Carisoprodol (Soma®)



Discriminative Stimulus Effects in Rhesus Monkeys (pentobarbital discrimination)

Monkeys responded >95% on the pentobarbital-associated lever after receiving the training dose (10.0 mg/kg, i.g.) of pentobarbital and responding exclusively on vehicle-associated lever after receiving saline or the CPDD-0054 vehicle, i.v. (Table 1). When given i.v. 5 minutes pre-session, CPDD-0054 engendered dose-related increases in pentobarbital-appropriate responding in all monkeys, achieving full substitution for pentobarbital in two of the three monkeys (Table 1). Sedation and anesthesia were observed immediately following i.v. injection in all monkeys with monkey Ef3 briefly anesthetized after the highest dose (30.0). This session was conducted 30 minutes after the injection.

Table 1. Discriminative stimulus effects of CPDD-0054 (i.v.) in monkeys discriminating between pentobarbital and vehicle.

	Subject					
	Ef3		8814		AQ63	
Drug (dose [mg/kg])	%DR	R/sec	%DR	R/sec	%DR	R/sec
Pentobarbital (10.0 i.g.)	100	1.72	100	1.33	96.5	1.63
Saline (i.g.)	0	2.33	0	1.65	0	2.10
Vehicle (i.v.)	0	2.62	0	1.68	0	2.64
CPDD-0054 (i.v.)						
(3.0)	5	2.15	0	1.36	0	2.85
(10.0)	0	2.23	50	1.33	82.5	2.34
(17.0)	84.5	1.26	n.s.	n.s.	96	2.35
(30.0)*	93	1.77	n.s.	n.s.	n.s.	n.s.

n.s. = not studied
* = session conducted 30 minutes post-injection
%DR = percentage of responses on the drug-appropriate lever
R/sec = responses (lever presses) per second

CPDD-0055
(-)-Phenylephrine hydrochloride

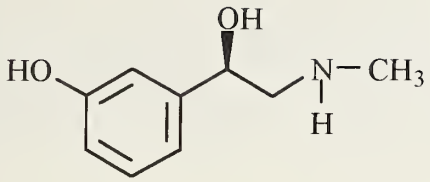


Table 2. Self-administration of cocaine, saline and CPDD-0055.

	Subject		
	UN	HO	BI
Drug (dose [mg/kg/inj])	R/sec	R/sec	R/sec
Cocaine (0.001)	0.10	0.21	0.15
(0.003)	0.77	0.63	0.16
(0.01)	1.55	1.15	0.60
(0.03)	1.50	0.88	1.15
Saline (1)	0.05	0.11	0.17
(2)	0.06	0.15	0.17
(3)	0.04	0.22	0.12
(4)	0.11	0.07	0.23
CPDD-0055			
(0.01)	0.06	0.10	0.14
(0.03)	0.05	0.32	0.27
(0.1)	0.11	0.14	0.11
(0.3)	0.02	0.03	0.03
(1.0)	0.02	0.02	0.06

n.s. = not studied
R/sec = responses (lever presses) per second
Inj = number of i.v. injections received during the session

Reinforcing Effects in Rhesus Monkeys

Cocaine self-administration (0.001–0.03 mg/kg/inj) increased rates of lever pressing in a dose-dependent manner with peak rates observed with the highest or second highest dose evaluated, whereas response-contingent saline did not maintain lever pressing. CPDD-0055 did not maintain rates of lever pressing at any dose tested (Table 2).

Discriminative Stimulus Effects in Rhesus Monkeys (*d*-amphetamine discriminations)

Monkeys that discriminated between saline and *d*-amphetamine responded 100% on the injection-appropriate lever during test sessions with the training drug or vehicle when the drugs were administered i.g. (60-minute pretreatment; Table 3) or i.m. (15-minute pretreatment; Table 4). Up to a dose of 30.0 mg/kg i.g., CPDD-0055 failed to substitute for *d*-amphetamine (Table 3) while having no systematic effect on rate of responding compared to vehicle. Similar results were obtained with i.m. administration of CPDD-0055 up to a dose of 17.0 mg/kg. Piloerection was exhibited by some monkeys following administration of the highest doses, regardless of route.

Table 3. Discriminative stimulus effects of CPDD-0055 (i.g.) in monkeys discriminating between *d*-amphetamine and vehicle.

	Subject					
	Ou3		8405		8515	
Drug (dose [mg/kg])	%DR	R/sec	%DR	R/sec	%DR	R/sec
d-Amphetamine (1.0)	100	1.76	100	1.56	100	2.89
Saline	0	2.26	0	2.19	0	2.34
CPDD-0055						
(3.0)	16	1.23	0	2.07	0	2.17
(10.0)	10	1.43	0	2.44	0	2.50
(30.0)	0	1.86	0	2.19	0	2.21

%DR = percentage of responses on the drug-appropriate lever
R/sec = responses (lever presses) per second

Table 4. Discriminative stimulus effects of CPDD-0055 (i.m.) in monkeys discriminating between *d*-amphetamine and vehicle.

	Subject							
	Ou3		8405		8515		M163*	
Drug (dose [mg/kg])	%DR	R/sec	%DR	R/sec	%DR	R/sec	%DR	R/sec
d-Amphetamine (1.0 i.m.)	100	2.00	100	1.56	100	2.28	94	0.24
Saline (i.m.)	0	2.02	0	2.55	0	2.49	5	0.31
CPDD-0055								
(1.0)	n.s.	n.s.	n.s.	n.s.	0	2.57	n.s.	n.s.
(3.0)	13	1.50	0	2.32	0	2.43	0	0.37
(10.0)	2	2.13	8	1.80	0	2.47	32	0.50
(17.0)	0	1.90	10	1.83	0	2.35	50	0.29

* = food-maintained responding
n.s. = not studied
%DR = percentage of responses on the drug-appropriate lever
R/sec = responses (lever presses) per second

Discriminative Stimulus Effects in Rhesus Monkeys (flumazenil discriminations)

In monkeys receiving 5.6 mg/kg/day of diazepam p.o. and discriminating between 0.32 mg/kg of flumazenil and vehicle, flumazenil produced dose-related increases in the percentage of responses emitted on the drug (flumazenil)-associated lever with a dose of 0.32 mg/kg occasioning greater than 80% drug-lever responding in all monkeys (Table 5). Under control (vehicle) conditions, the average rates of responding for the three monkeys used in this study were 1.33 $\pm$ 0.16 (DU), 1.11 $\pm$ 0.13 (DA) and 1.48 $\pm$ 0.11 (CR) responses per second. The largest dose of flumazenil decreased response rates to less than 50% of control (not shown).

Up to a dose of 10.0 or 32.0 mg/kg, CPDD 0055 failed to substitute (i.e., did not produce at least 80% drug-lever responding) for the flumazenil discriminative stimulus (Table 5). One monkey (DA) responded a maximum of 28.9% on the flumazenil-associated lever at a dose of 3.2 mg/kg of CPDD 0055. A dose of 10.0 mg/kg of CPDD 0055 decreased response rates to less than 10% of control in two monkeys (DA and CR) and a dose of 32.0 mg/kg decreased rate to less than 60% of control in a third monkey (not shown).

TABLE 5. Discriminative stimulus effects of flumazenil and CPDD-0055 in diazepam-treated monkeys discriminating between flumazenil and vehicle.

	Subject		
	DU	DA	CR
Drug (dose [mg/kg])	%DR	%DR	%DR
Vehicle	0	0	0
Flumazenil			
(0.01)	0	0	0
(0.032)		3.7	29.4
(0.1)	0	20.0	31.4
(0.32)	96.2	96.2	100
Vehicle	0	0	0
CPDD-0055			
(1.0)	0	0	0
(3.2)	0	28.9	0
(10.0)	0	25.0	0
(32.0)	0	n.s.	n.s.

n.s. = not studied
%DR = percentage of responses on the drug-appropriate lever

TABLE 6. Discriminative stimulus effects of midazolam and CPDD-0055 in monkeys discriminating between midazolam and vehicle.

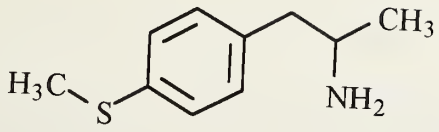
	Subject	
	MA	RO
Drug (dose [mg/kg])	%DR	%DR
Vehicle	0	
Midazolam		
(0.032)	10.0	0
(0.1)	0	0
(0.32)	100	100
Vehicle	0	0
CPDD-0055		
(1.0)	0	0
(3.2)	0	0
(10.0)	0	0
(32.0)	0	0

%DR = percentage of responses on the drug-appropriate lever

Discriminative Stimulus Effects in Rhesus Monkeys (midazolam discriminations)

In monkeys discriminating between 0.56 mg/kg of midazolam and vehicle, midazolam produced dose-related increases in the percentage of responses emitted on the drug (midazolam)-associated lever with a dose of 0.32 mg/kg occasioning greater than 80% drug-lever responding in both monkeys (Table 6). Under control conditions, the average rates of responding for the two monkeys used in this study were 1.78 $\pm$ 0.13 (MA) and 1.21 $\pm$ 0.05 (RO) responses per second. Over the doses studied, midazolam slightly decreased rates of responding (not shown). Up to a dose of 32.0 mg/kg s.c., CPDD 0055 failed to substitute for midazolam in either monkey (Table 6) and did not systematically alter rates of responding (not shown).

CPDD-0056
4-Methylthioamphetamine hydrochloride



Reinforcing Effects in Rhesus Monkeys

Cocaine self-administration (0.001–0.03 mg/kg/inj) increased rates of lever pressing in a dose-dependent manner with peak rates observed with the highest or second highest dose evaluated, whereas response-contingent saline did not maintain lever pressing. CPDD-0056 did not maintain rates of lever pressing at any dose tested.

Table 7. Self-administration of cocaine, saline and CPDD-0056.

	Subject		
	UN	HO	BI
Drug (dose [mg/kg/inj])	R/sec	R/sec	R/sec
Cocaine (0.001)	0.23	0.28	0.19
(0.003)	0.29	1.35	0.17
(0.01)	1.41	1.44	0.78
(0.03)	1.25	1.21	1.23
Saline (1)	0.06	0.08	0.03
(2)	0.08	0.32	0.04
(3)	0.05	0.06	0.06
(4)	0.13	0.09	0.26
CPDD-0056			
(0.01)	0.04	0.29	0.09
(0.03)	0.21	0.27	0.12
(0.1)	0.15	0.20	0.18
(0.3)	0.08	0.01	0.26
(1.0)	0.07	0.03	0.01

n.s. = not studied
R/sec = responses (lever presses) per second
Inj = number of i.v. injections received during the session

Table 8. Discriminative stimulus effects of CPDD-0056 (i.g.) in monkeys discriminating between *d*-amphetamine and vehicle.

	Subject					
	Ou3		8405		8515	
Drug (dose [mg/kg])	%DR	R/sec	%DR	R/sec	%DR	R/sec
d-Amphetamine (1.0)	100	1.76	100	1.88	100	2.89
Saline	0	2.26	0	2.19	0	2.34
CPDD-0056						
(1.0)	0	1.55	0	2.66	0	2.21
(3.0)	0	1.74	0	2.35	0	2.26

%DR = percentage of responses on the drug-appropriate lever
R/sec = responses (lever presses) per second

Table 9. Discriminative stimulus effects of CPDD-0056 (i.m.) in monkeys discriminating between *d*-amphetamine and vehicle.

	Subject							
	Ou3		8405		8515		M163*	
Drug (dose [mg/kg])	%DR	R/sec	%DR	R/sec	%DR	R/sec	%DR	R/sec
d-Amphetamine (1.0 i.m.)	100	2.00	100	2.55	100	2.28	94	0.24
Saline (i.m.)	0	2.02	0	1.56	0	2.49	5	0.31
CPDD-0056								
(0.3)	8	1.47	n.s.	n.s.	0	2.46	0	0.30
(0.056)	n.s.	n.s.	0	2.37	n.s.	n.s.	n.s.	n.s.
(1.0)	94	1.17	98	1.44	0	2.25	0	0.24
(3.0)	n.s.	n.s.	n.s.	n.s.	0	1.69	0	0.00†

* = food-maintained discrimination
n.s. = not studied
† = no responding
%DR = percentage of responses on the drug-appropriate lever
R/sec = responses (lever presses) per second

Discriminative Stimulus Effects in Rhesus Monkeys (*d*-amphetamine discrimination)

Monkeys that discriminated between saline and either *d*-amphetamine (either via i.g. or i.m. administration) responded at or near 100% on the injection-appropriate lever during test sessions with the training drug or vehicle (Tables 8 and 9). Up to a dose of 3.0 mg/kg, i.g., CPDD-0056 failed to substitute for *d*-amphetamine while having no systematic effect on rate of responding (Table 8). However, when administered via the i.m. route, CPDD-0056 (1.0 mg/kg) substituted for the training drug in two monkeys (Table 9). Response rates were not systematically affected.

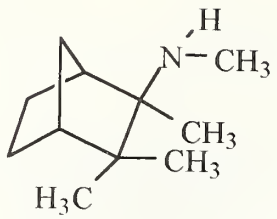
A higher dose (3.0 mg/kg, i.m.) was tested in two of the monkeys but did not substitute for the training stimulus. Following administration of this dose, one subject (8515) exhibited slow, but repetitive head and eye movements, did not consume treats, and was relatively inactive. The other subject (M163) did not respond during the session and was observed to be hypervigilent and excitable, did not consume treats, and exhibited stereotypy, e.g., repetitive head movements, picking at skin/pulling at fur. These effects lasted at least two hours following drug administration. Higher doses were not evaluated due to concern for the animals' health.

Discriminative Stimulus Effects in Rhesus Monkeys (midazolam discrimination)

In monkeys discriminating between 0.56 mg/kg of midazolam and vehicle, midazolam produced dose-related increases in the percentage of responses emitted on the drug (midazolam)-associated lever with a dose of 0.32 mg/kg occasioning greater than 80% drug-lever responding in all monkeys (not shown).

Acute administration of CPDD-0056, up to a dose of 3.2 mg/kg, failed to occasion any midazolam-lever responding and did not affect rates of responding. When 10.0 mg/kg of CPDD-0056 was administered, the female monkey (FR) failed to respond during either of two cycles; consequently, the test session was terminated. Subsequently the monkey appeared sedated, displayed mydriasis, later showed little muscle tone, did not respond to tactile stimulation, and respiration was infrequent and irregular. These signs appeared to remain stable for 3-5 hours after drug administration. The monkey expired approximately 6.5 hrs after receiving 10.0 mg/kg of CPDD-0056. Necropsy revealed pulmonary edema and hemorrhaging in both caudal lobes. No further experiments were conducted with CPDD-0056.

CPDD-0057
S-(+)-Mecamylamine hydrochloride



Reinforcing Effects in Rhesus Monkeys

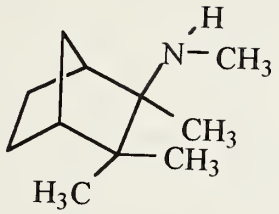
Methohexital (0.1 mg/kg/inj) maintained responding at rates greater than observed for saline administration. CPDD-0057 (0.01-0.1 mg/kg/inj) did not maintain responding at levels above saline rates (Table 10). Due to post-session behavioral changes with monkey JZ it was concluded that behaviorally active doses were being tested.

Table 10. Self-administration of methohexital, saline and CPDD-0057.

	Subject					
	RG		RS		JZ	
Drug (dose [mg/kg/inj])	R/sec	Inj	R/sec	Inj	R/sec	Inj
Methohexital (0.1)	0.14	88	0.13	88	0.17	106
Saline	0.01	7	0.62	16	0.02	12
CPDD-0057						
(0.01)	0.01	4	0.29	16	0.02	16
(0.03)	0.01	10	0.02	16	0.02	14
(0.1)	0.01	7	0.03	16	0.03	22

n.s. = not studied
R/sec = responses (lever presses) per second
Inj = number of i.v. injections received during the session

CPDD-0058
R-(-)-Mecamylamine hydrochloride



Reinforcing Effects in Rhesus Monkeys

Methohexital (0.1 mg/kg/inj) maintained responding at rates greater than observed for saline administration. CPDD-0057 (0.01-0.1 mg/kg/inj) did not maintain responding at levels above saline rates (Table 11). Due to post-session behavioral changes with monkey RS it was concluded that behaviorally active doses were being tested.

Table 11. Self-administration of methohexital, saline and CPDD-0058.

	Subject					
	RG		RS		PC	
Drug (dose [mg/kg/inj])	R/sec	Inj	R/sec	Inj	R/sec	Inj
Methohexital (0.1)	0.13	91	0.01	68	0.28	151
Saline	0.01	7	0.01	9	0.01	7
CPDD-0058						
(0.01)	0.01	10	0.02	15	0.01	7
(0.03)	0.01	6	0.02	13	0.01	6
(0.1)	0.15	10	0.03	19	0.01	7
(0.3)	0.01	5	0.01	9	0.02	12

n.s. = not studied
R/sec = responses (lever presses) per second
Inj = number of i.v. injections received during the session

CONCLUSIONS

CPDD-0054

It was previously reported (France et al., 1999) that CPDD-0054 (carisoprodol) maintained i.v. self-administration responding that was greater than rates maintained by saline, although less than rates maintained by i.v. injections of methohexital. Solubility limits precluded studies on doses of CPDD-0054 larger than 0.3 mg/kg/injection. CPDD-0054 (3.2-32.0 mg/kg, s.c.) also failed to substitute for the flumazenil discriminative stimulus in diazepam-treated monkeys or for the midazolam discriminative stimulus in untreated monkeys. In addition, CPDD-0054 did not substitute for pentobarbital when administered by the i.g. route. However, new work presented here shows that CPDD-0054 substituted for pentobarbital when administered by the i.v. route. Notably, this effect was accompanied by sedation and anesthesia at the highest dose. Lack of pentobarbital-like effects following i.g. administration may have been due to poor absorption after i.g. administration.

CPDD-0055

CPDD-0055 did not maintain rates of responding indicative of a reinforcing effect. When CPDD-0055 was made available for i.v. self-administration in the morning, between 10:00 and 12:00, rates of behavior maintained by cocaine at 4:00 that afternoon were suppressed. This suggests that active doses of CPDD-0055 were made available in this situation. CPDD-0055 does not appear to have any reinforcing effect in rhesus monkeys under the circumstances described here.

CPDD-0055 did not substitute for *d*-amphetamine up to doses that were observed to produce other effects, e.g., piloerection. These effects occurred following the administration of the highest doses evaluated via two different routes of administration, i.e., 30 mg/kg, i.g. and 17 mg/kg, i.m. Therefore, these results suggest that CPDD-0055 does not have *d*-amphetamine-like discriminative stimulus effects in rhesus monkeys.

CPDD-0055 failed to substitute for flumazenil in diazepam-treated monkeys and also failed to substitute for midazolam in otherwise untreated monkeys. While it is possible that CPDD-0055 might have discriminative stimulus effects under conditions different from those used in the current study, for doses up to and including 32.0 mg/kg, CPDD-0055 fails to exert either benzodiazepine antagonist-like or benzodiazepine agonist-like discriminative stimulus effects in rhesus monkeys.

CPDD-0056

CPDD-0056 did not maintain rates of responding indicative of a reinforcing effect. Animals were observed remotely during sessions of CPDD-0056 availability and there was no evidence that the drug produced any direct effects on behavior. Larger doses were not evaluated due to the fact that the two largest does tested produced markedly low rates of responding. This suggests that if larger doses had been evaluated, responding would not have been maintained. Therefore, it is likely that CPDD-0056 does not any reinforcing effect in rhesus monkeys.

CPDD-0056 when given by the i.g. route did not substitute for *d*-amphetamine. However, when the i.m. route of administration was employed, CPDD-0056 (1.0 mg/kg) substituted for *d*-amphetamine in two of the four subjects tested. Higher doses were not tested due to concern for the animals' health. However, behaviorally active doses were suggested by the animals' post-session behavior (e.g. stereotypy) following the highest dose evaluated (3.0 mg/kg, i.m.). Thus, CPDD-0056 may have some *d*-amphetamine-like discriminative stimulus effects.

CPDD-0056 failed to substitute for midazolam up to a dose of 3.2 mg/kg, s.c. A larger dose was lethal in one monkey and no further tests were conducted.

CPDD-0057

No dose of CPDD-0057 tested maintained responding above levels maintained by saline. Therefore, CPDD-0057 does not appear to have any reinforcing effect in rhesus monkeys under the circumstances described here.

CPDD-0058

No dose of CPDD-0058 tested maintained responding above levels maintained by saline. Therefore, CPDD-0058 does not appear to have any reinforcing effect in rhesus monkeys under the circumstances described here.

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ACKNOWLEDGEMENTS

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EVALUATION OF NEW COMPOUNDS FOR OPIOID ACTIVITY (2000)

J.H. Woods and J.R. Traynor

Department of Pharmacology, University of Michigan, Ann Arbor, MI

This report contains information on opioid abuse liability evaluations on compounds that have been submitted to the Drug Evaluation Committee of the College and released for publication by the submitters. The information obtained usually involves *in vitro* evaluation in opioid binding assays. In addition, the compounds may be evaluated for discriminative and reinforcing effects. Analgesic and respiratory function assays are also possible. These behavioral assessments are conducted in rhesus monkeys (see Appendix). Usually when limited information is provided (*e.g.*, *in vitro* assessment only), it is because the sample provided by the submitter was insufficient to carry out further evaluation.

The evaluation of new compounds by the programs at the University of Michigan and the Medical College of Virginia was coordinated by Dr. Arthur E. Jacobson, Laboratory of Medicinal Chemistry, NIDDK, National Institutes of Health, Bethesda, MD, and is currently coordinated by Dr. A. Coop, University of Maryland. The compounds, which come originally from pharmaceutical companies, universities, government laboratories, and international organizations are now submitted to Dr. Coop.

At the UM and MCV laboratories, drug samples arrive from the Biological Coordinator with only the following information: (1) an identifying NIH number, (2) molecular weight, (3) solubility information, and (4) a recommended starting dose. After the evaluation is complete and the report submitted to Dr. Coop, the submitter is requested to release the chemical structure to include with the evaluation data in the ANNUAL REPORT. The submitter has up to three years before release of the structure is required. When the structure is released all of the data on the compound are reported herein.

OPIOID RECEPTOR BINDING AND IN VITRO EFFICACY ASSESSMENT

Details of the binding assay been described previously (Lee et al., 1999). Briefly, aliquots of a membrane preparation are incubated with [^{3}H]diprenorphine (0.3 nM) in the presence of different concentrations of the drug under investigation at 25° C for 1 hr. Specific, *i.e.*, opioid-receptor-related binding is determined as the difference in binding obtained in the absence and presence of 10μM naloxone. The potency of the drugs in displacing the specific binding of ^{3}H-ligand is determined from data using Graphpad Prism (GraphPAD, San Diego, CA) and converted to Ki values by the method of Cheng and Prussoff (1973). Mu and δ binding were performed in membranes from C_6 rat glioma cells expressing recombinant μ (rat; Emmerson et al., 1994) or δ (rat; Clark et al., 1997) and CHO cells expressing the recombinant κ (human, Zhe et al., 1997). The affinity (Kd) values of [^{3}H]diprenorphine at the receptors are: μ (0.15 nM); δ (0.45 nM); κ (0.25 nM).

This year, our assays all use recombinant receptors rather than homogenates of monkey brain cortex. The use of recombinant receptors means no cross-reaction with other receptors and allows for direct comparison with cellular functional assays. In the **ANNUAL REPORT**, the results of the selective binding assays are given as means ± SEM from three separate experiments, each performed in duplicate. Ki values for standard compounds using recombinant receptors and [^{3}H]diprenorphine as radioligand are: μ (DAMGO, 7.6 nM; morphine, 11.2 nM), δ (SNC80, 0.8 nM) and κ (U69593, 0.3 nM).

[^{35}S]GTPγS assays are carried out using membranes from C6 cells expressing either μ (Emmerson et al., 1996) or δ (Clark et al., 1997) receptors or CHO cells expressing κ receptors (Zhu et al., 1997). Assays are performed as described by Traynor and Nahorski (1995). Values are given as EC_{50} with % effect compared to standard agonist

(DAMGO, SNC80, or U69593) or as maximal stimulation achieved at 3 μM.

EC_{50} values (nM) for standard compounds are as follows:

Mu receptor: morphine (65), DAMGO (34), fentanyl (13)
Delta receptor: SNC80 (9), DPDPE (8.3)
Kappa receptor: U69593 (31.0), bremazocine (0.5)

DPDPE (60%) and bremazocine (86%) are partial agonists compared with the standards SNC80 and U69593. Morphine and DAMGO give equivalent responses.

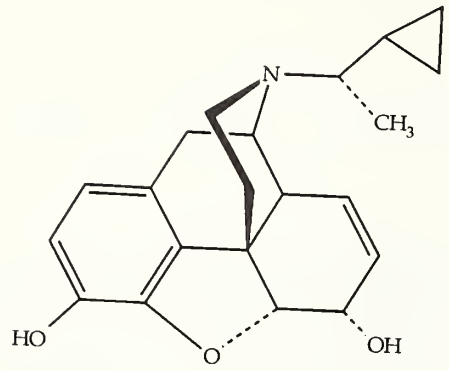
SUMMARY OF TESTS PERFORMED

The compounds which were evaluated at the University of Michigan during the past year, and the individual tests which were performed are shown in the following Table. Also shown are dates of Reports to the Biological Coordinator, Dr. A.E. Jacobson or Dr. Coop, in which results are reported.

NIH #	Date Submitted to Biological Coordinator	NIH #	Date Submitted to Biological Coordinator
10497†		10976	22 September 1999
10963*	5 April 1999	10977	22 September 1999
10964*	5 April 1999	10980	22 September 1999
10965*	5 April 1999	10981	22 September 1999
10966*	5 April 1999	10982	22 September 1999
10968*	5 April 1999	10983	28 February 2000
10969*	5 April 1999	10994	28 February 2000
10970	5 April 1999	10993	28 February 2000
10971	5 April 1999	10998	28 February 2000
10972	5 April 1999	10999	7 March 2000
10973	5 April 1999	11000	7 March 2000
10974	5 April 1999	11001	7 March 2000
10975	5 April 1999	11002	7 March 20000

* [^{3}H]DAMGO used to label μ sites
† Other data on 10497 has been reported in the 1989 Annual Report

NIH 10497 N-(1*R*-1-Cyclopropyl)ethylnormorphine hydrochloride



[^{35}S]GTPγS BINDING
EC_{50} (Maximal Stimulation)

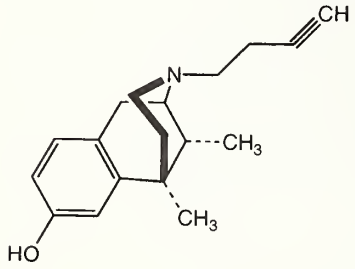
μ-receptor: 2191 ± 773 (18.7 ± 5.8)
δ-receptor: 72.2 ± 21 (11.7 ± 3.3)
κ-receptor: 18.3 ± 4.1 (78.4 ± 3.8)

SUMMARY

NIH 10497 was a κ agonist.

* * *

NIH 10963 (±)-2-(3-Butynyl)-5-9α-dimethyl-2'-hydroxy-6,7-benzomorphan[(±)-N-(3-Butynyl)-N-normetazocine]



OPIOID RECEPTOR BINDING (nM)

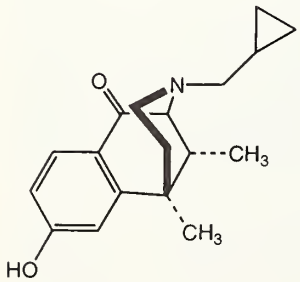
μ-receptor:	0.79 ± 0.08
δ-receptor:	14.7 ± 2.2
κ-receptor:	3.0 ± 0.2

SUMMARY

NIH 10963 had high affinity for all three opioid receptors, with some selectivity for μ over κ (4-fold) and μ over δ (19-fold).

* * *

NIH 10964 (±)-8-Ketocyclazocine
(2-Cyclopropylmethyl-5,9α-dimethyl-2'-hydroxy-8-keto-6,7-benzomorphan)



OPIOID RECEPTOR BINDING (nM)

μ-receptor:	6.0 ± 0.8
δ-receptor:	6.9 ± 0.6
κ-receptor:	4.0 ± 0.9

NIH 10964 (continued)

[^{35}S]GTPγS BINDING
EC_{50} (Maximal Stimulation)

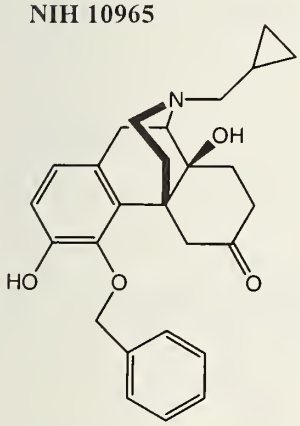
μ-receptor: 273 ± 103 (19.1 ± 4.9)
δ-receptor: 122 ± 35 (45.9 ± 9.6)
κ-receptor: 14.3 ± 1.6 (91.0 ± 4.3)

SUMMARY

NIH 10964 had good affinity for μ, κ and δ opioid receptors, with no selectivity. It was a strong agonist at only the κ receptor.

* * *

NIH 10965 **4-Benzyloxy-17-cyclopropylmethyl-14-hydroxy-17-nordihydrothebainone**



OPIOID RECEPTOR BINDING (nM)

μ-receptor:	10.4 ± 0.8
δ-receptor:	6476 ± 1506
κ-receptor:	202 ± 11.2

[^{35}S]GTPγS BINDING
EC_{50} (Maximal Stimulation)

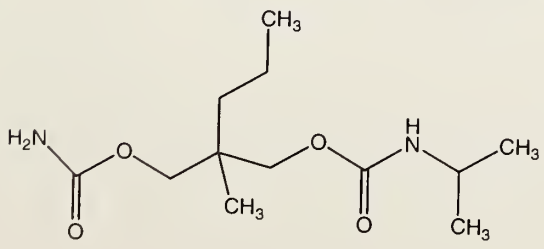
μ-receptor: 1254 ± 576 (21.4 ± 10.6)

SUMMARY

NIH 10965 had good affinity for μ receptors and 600-fold selectivity for μ over δ receptors, and 20-fold selectivity for μ over κ receptors. It had a very weak μ-agonist effect.

* * *

NIH 10966 **Carisoprodol**
((1-Methylethyl)carbamic acid-[[(aminocarbonyl)oxy]methyl]-2-methylpentyl ester)



OPIOID RECEPTOR BINDING (μM)

μ-receptor:	>10 (11 ± 1.5% inhibition at 10 μM)
δ-receptor:	>10 (1.7 ± 1.7% inhibition at 10 μM)
κ-receptor:	>10 (1.7 ± 0.9% inhibition at 10 μM)

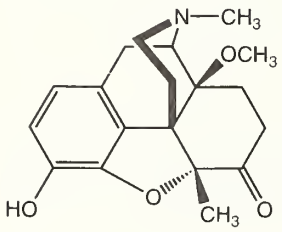
NIH 10966 (continued)

SUMMARY

NIH 10966 had no affinity for opioid receptors.

* * *

NIH 10968 **4-Methoxy Metopon**
(-)-4,5α-Epoxy-3-hydroxy-14-methoxy-5,17-dimethylmorphinan-6-one hydrobromide



OPIOID RECEPTOR BINDING (nM)

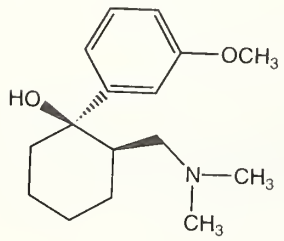
μ-receptor:	0.023 ± 0.008
δ-receptor:	40.9 ± 7.4
κ-receptor:	304 ± 52

SUMMARY

NIH 10968 had extremely high affinity and selectivity (δ/μ = 1800-fold; κ/μ = 9000-fold) for μ receptors.

* * *

NIH 10969 **Tramadol hydrochloride**
***trans*-(±)-2-[(Dimethylamino)methyl]-1-(3-methoxyphenyl)cyclohexanol hydrochloride**



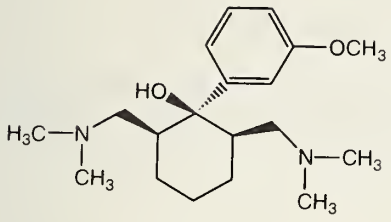
OPIOID RECEPTOR BINDING (nM)

μ-receptor:	2995 ± 661
δ-receptor:	>10 μM (7.7 ± 3.2% inhibition at 10 μM)
κ-receptor:	>10 μM (9.3 ± 3.0% inhibition at 10 μM)

SUMMARY

NIH 10969 had low affinity for the μ receptor and even lower affinity for δ and κ receptors.

NIH 10970 **2,6-Bis[(dimethylamino)methyl]-1-(3-methoxyphenyl)cyclohexanol dihydrochloride**



OPIOID RECEPTOR BINDING (μM)

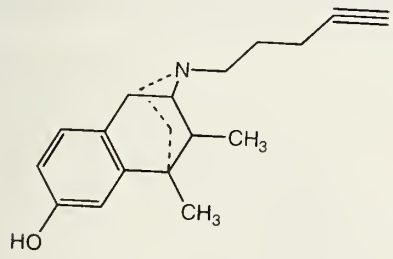
μ-receptor: >10 (1.3 ± 4.9% inhibition at 10 μM)
δ-receptor: >10 (-3.7 ± 1.8% inhibition at 10 μM)
κ-receptor: >10 (4.3 ± 2.4% inhibition at 10 μM)

SUMMARY

NIH 10970 had no affinity for opioid receptors.

* * *

NIH 10971 **(+)-(1*S*,5*S*,9*S*)-5,9-dimethyl-2'-hydroxy-2-(4-pentynyl)-6,7-benzomorphan**



OPIOID RECEPTOR BINDING (nM)

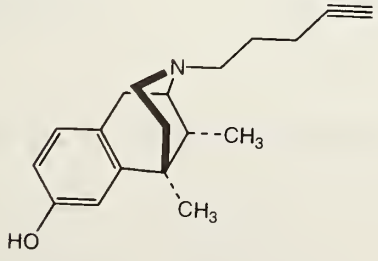
μ-receptor:	3572 ± 682
δ-receptor:	>10 μM (2.3 ± 14.9% inhibition at 10 μM)
κ-receptor:	328 ± 35.6

SUMMARY

NIH 10971 had low affinity for the κ receptor, but did show selectivity for this receptor over μ receptors (10-fold) and δ receptors (>30-fold).

* * *

NIH 10972 **(-)-(1*R*,5*R*,9*R*)-5-9-dimethyl-2'-hydroxy-2-(4-pentynyl)-6,7-benzomorphan**



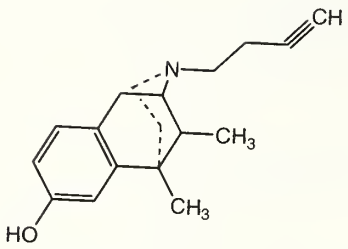
OPIOID RECEPTOR BINDING (nM)

μ-receptor:	17.6 ± 3.9
δ-receptor:	137 ± 26
κ-receptor:	11.1 ± 2.4

SUMMARY

NIH 10972 had affinity for all the opioid receptors, with higher affinity for μ and κ receptors than the δ receptor.

NIH 10973 **(+)-(1*S*,5*S*,9*S*)-2-(3-Butynyl)-5,9-dimethyl-2'-hydroxy-6,7-benzomorphan hydrochloride**



OPIOID RECEPTOR BINDING (nM)

μ-receptor: >10 μM (36.3 ± 3.2% inhibition at 10 μM)
δ-receptor: >10 μM (14.0 ± 3.5% inhibition at 10 μM)
κ-receptor: 1726 ± 406

SUMMARY

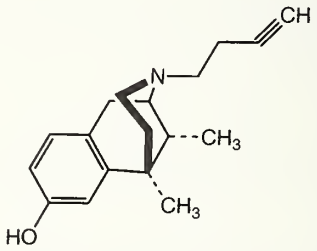
NIH 10973 had very low affinity for μ and δ opioid receptors, with slightly improved -- but still very low -- affinity for κ receptors.

* * *

NIH 10974 **(-)-(1*R*,5*R*,9*R*)-2-(3-Butynyl)-5,9-dimethyl-2'-hydroxy-6,7-benzomorphan hydrochloride**

OPIOID RECEPTOR BINDING (nM)

μ-receptor: 4.6 ± 0.5
δ-receptor: 9.9 ± 1.6
κ-receptor: 1.4 ± 0.1



SUMMARY

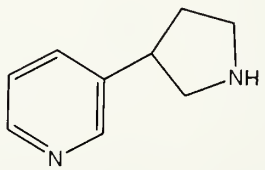
NIH 10974 had high affinity for μ, κ and δ opioid receptors.

* * *

NIH 10975 **(-)-N-Norisonicotine di-l-tartrate**

OPIOID RECEPTOR BINDING (μM)

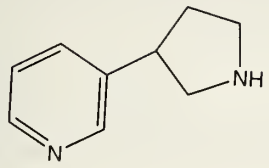
μ-receptor: >10 (19.0 ± 5.2% inhibition at 10 μM)
δ-receptor: >10 (9.0 ± 4.7% inhibition at 10 μM
κ-receptor: >10 (43.3 ± 0.6%) inhibition at 10 μM)



SUMMARY

NIH 10975 had no affinity for opioid receptors.

NIH 10976 **(+)-N-Norisonicotine di-d-tartrate**



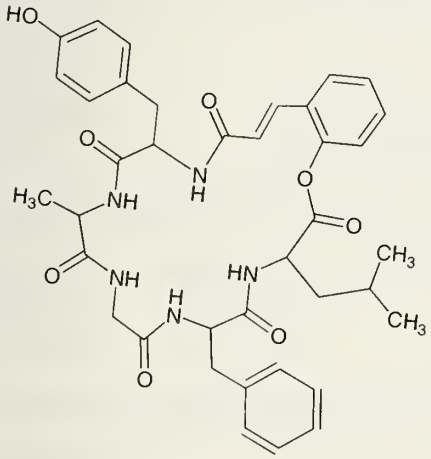
OPIOID RECEPTOR BINDING (μM)

μ-receptor: >10 (10.0 ± 1.2% inhibition at 10 μM)
δ-receptor: >10 (4.3 ± 4.3% inhibition at 10 μM)
κ-receptor: >10 (7.7 ± 2.5%) inhibition at 10 μM)

SUMMARY

NIH 10976 had no affinity for μ, δ and κ opioid receptors.

NIH 10977 **Coumarin-based cyclic prodrug of DADLE**



OPIOID RECEPTOR BINDING (μM)

μ-receptor: >10 (14.3 ± 5.1% inhibition at 10 μM)
δ-receptor: >10 (31.0 ± 6.4% inhibition at 10 μM)
κ-receptor: >10 (7.2 ± 4.3%) inhibition at 10 μM)

SUMMARY

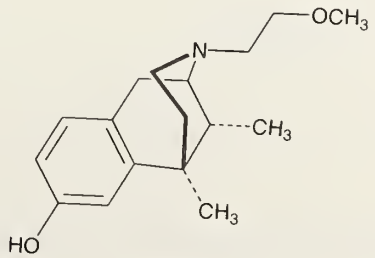
NIH 10977 had very low affinity for μ, δ and κ opioid receptors.

* * *

NIH 10980 **(-)-(1*R*,5*R*,9*R*)-5,9-Dimethyl-2'-hydroxy-2-(2-methoxyethyl)-6,7-benzomorphan hydrochloride**

OPIOID RECEPTOR BINDING (nM)

μ-receptor:	0.48 ± 0.13
δ-receptor:	8.5 ± 0.8
κ-receptor:	0.84 ± 0.27



SUMMARY

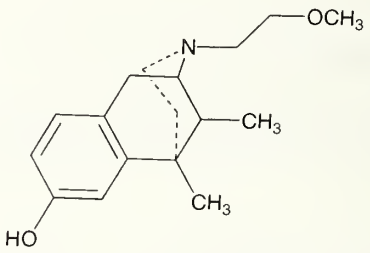
NIH 10980 had excellent μ and κ affinity and does not select between these receptors. It has 10-18 times lower affinity at the δ receptor, though this still represents good δ binding.

* * *

NIH 10981 **(+)-(1*S*,5*S*,9*S*)-5,9-Dimethyl-2'-hydroxy-2-(2-methoxyethyl)-6,7-benzomorphan hydrochloride**

OPIOID RECEPTOR BINDING (nM)

μ-receptor:	778 ± 158
δ-receptor:	5712 ± 222
κ-receptor:	1158 ± 333

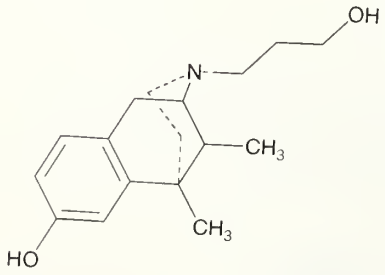


SUMMARY

NIH 10981 had low affinity at all three opioid receptors in the order μ > κ > δ

* * *

NIH 10982 **(+)-(1*S*,5*S*,9*S*)-5,9-Dimethyl-2'-hydroxy-2-(3-hydroxypropyl)-6,7-benzomorphan hydrochloride**



OPIOID RECEPTOR BINDING (nM)

μ-receptor:	2102 ± 245
δ-receptor:	>10 μM (22.7 ± 1.5% at 10 μM)
κ-receptor:	915 ± 294

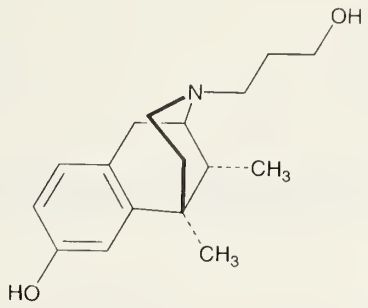
SUMMARY

NIH 10982 had low affinity at all three opioid receptors in the order κ > μ > δ.

NIH 10983 **(-)-(1*R*,5*R*,9*R*)-5,9-Dimethyl-2'-hydroxy-2-(3-hydroxypropyl)-6,7-benzomorphan hydrochloride**

OPIOID RECEPTOR BINDING (nM)

μ-receptor: 34.0 ± 5.1
δ-receptor: 124 ± 8.8
κ-receptor: 137 ± 74



SUMMARY

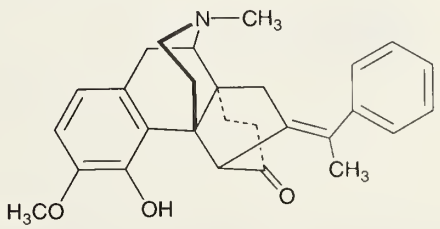
NIH 10983 had affinity for opioid receptors in the order μ > κ = δ.

* * *

NIH 10984 **E-3-Methoxy-4-hydroxy-5,14-ethano-18-(1-methyl)benzylidene-6-oxo-N-methylmorphinan**

OPIOID RECEPTOR BINDING (nM)

μ-receptor: 0.92 ± 0.06
δ-receptor: 6.7 ± 1.0
κ-receptor: 0.42 ± 0.07



SUMMARY

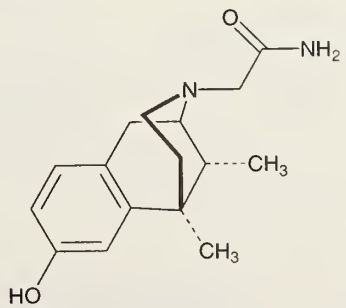
NIH 10984 had high affinity for κ and μ opioid receptors and approximately 10-fold less (but still high) affinity for the δ opioid receptors.

* * *

NIH 10993 **(-)-(1*R*,5*R*,9*R*)-2-Acetamido-5,9-dimethyl-2'-hydroxy-6,7-benzomorphan**

OPIOID RECEPTOR BINDING (nM)

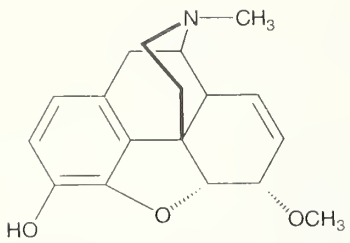
μ-receptor: 677 ± 86
δ-receptor: 2005 ± 313
κ-receptor: 304 ± 48



SUMMARY

NIH 10993 had some affinity for κ > μ opioid receptors, but poor affinity at the δ receptor.

NIH 10998 **Heterocodeine hydrochloride**



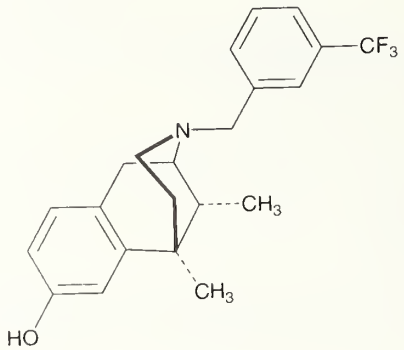
OPIOID RECEPTOR BINDING (nM)

μ-receptor:	20.8 ± 1.4
δ-receptor:	251 ± 9.9
κ-receptor:	271 ± 43

SUMMARY

NIH 10998 had good affinity for the μ opioid receptor and was approximately 12-fold selective for this receptor over the δ and κ receptors.

NIH 10999 **(-)-(1*R*,5*R*,9*R*)-5,9-Dimethyl-2'-hydroxy-2-(3-trifluoromethylbenzyl)-6,7-benzomorphan hydrochloride**



OPIOID RECEPTOR BINDING (nM)

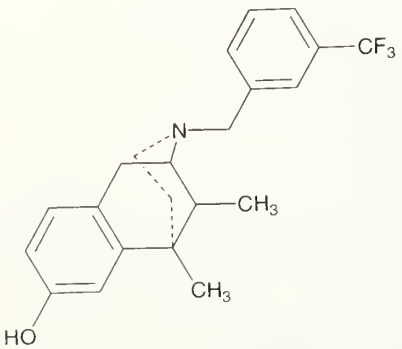
μ-receptor:	314 ± 75.6
δ-receptor:	2904 ± 414
κ-receptor:	704 ± 111

SUMMARY

NIH 10999 has poor affinity for opioid receptors in the order μ > κ > δ.

* * *

NIH 11000 **(+)-(1S,5S,9S)-5,9-Dimethyl-2'-hydroxy-2-(3-trifluoromethylbenzyl)-6,7-benzomorphan hydrochloride**



OPIOID RECEPTOR BINDING (nM)

μ-receptor:	598 ± 166
δ-receptor:	1644 ± 502
κ-receptor:	528 ± 37

NIH 11000 (continued)

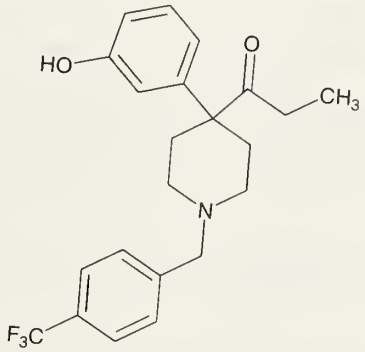
SUMMARY

NIH 11000 binds weakly to opioid receptors in the order μ = κ > δ.

* * *

NIH 11001 **4-(3-Hydroxyphenyl)-4-(1-oxopropyl)-1-(4-trifluoromethylbenzyl) piperidine hydrochloride**

OPIOID RECEPTOR BINDING (nM)



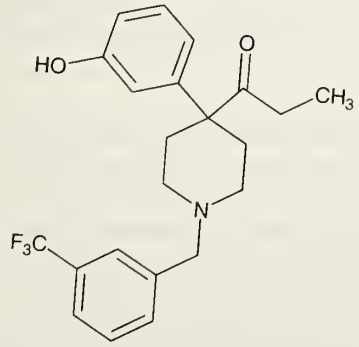
μ-receptor:	32.9 ± 1.1
δ-receptor:	291 ± 83
κ-receptor:	118 ± 28

SUMMARY

NIH 11001 had reasonable affinity for the μ opioid receptor > κ = δ.

* * *

NIH 11002 **4-(3-Hydroxyphenyl)-4-(1-oxopropyl)-1-(3-trifluoromethylbenzyl) piperidine hydrochloride**



OPIOID RECEPTOR BINDING

μ-receptor:	118 ± 28
δ-receptor:	316 ± 32
κ-receptor:	203 ± 33

SUMMARY

NIH 11002 had some affinity for the μ, δ and κ receptors, but was non-selective.

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APPENDIX

University of Michigan laboratories also offer the following tests:

DRUG DISCRIMINATION IN RHESUS MONKEYS

We currently use three groups of monkeys to test the discriminative stimulus effects of submitted drugs: one of these groups discriminates the administration of the κ agonist ethylketazocine (EKC); a second group discriminates the μ agonist alfentanil or fentanyl; a third group is treated daily with morphine and discriminates the opioid antagonist naltrexone.

The procedures used with the EKC-trained monkeys have been described by Bertalmio et al. (1982). The monkeys are removed from their home cages each day and seated in primate restraining chairs. These chairs are placed in chambers equipped with two response levers, several stimulus lights and a cup to receive Noyes, banana-flavored pellets. These monkeys are required to make 100 consecutive responses on the correct one of the two levers and receive ten 300-mg food pellets. The right lever is correct if they were given a subcutaneous injection of 0.0032 mg/kg EKC immediately prior to the start of the cycle. The left lever is designated correct if they were given a sham injection before the start of the cycle. Each cycle lasts 15-min and consists of an initial 10-min black out period followed by a period of as long as 5 min, during which a blue light is illuminated in the chamber and the monkey can respond for food. If the food pellets are delivered before the 5 min period is completed, the lights are extinguished for the remainder of this time. Typically, a daily session consists of several 15 min cycles. During a training session, if EKC is given, it is given on the penultimate cycle of that session. Responding on the drug-appropriate lever is reinforced during that cycle and on the subsequent, final cycle of the day. These last two cycles may be preceded by from zero to four sham cycles on a training day. A training session of six sham cycles is also scheduled from time to time.

With this type of multiple, discrete-cycle training, the animals can be tested with a cumulative dosing procedure. On a test session, the first cycle is preceded by an injection of saline, and prior to subsequent cycles, increasing, cumulative doses of the test drug are administered. One hundred consecutive responses on either lever are reinforced throughout the test session. The test drug is administered in increasing doses until the monkey either responds on the drug-appropriate lever, the response rate falls to less than half of the saline-control rate, or six cycles are given. In the latter situation, it is assumed that the selected dose range is too low, and the test is continued at higher doses on the next test session. Each test session is preceded and followed by a training session. The criterion for satisfactory performance must be met on each training session that is followed by a test session. This criterion is that at least 90% of the responses during each cycle of a training session must be on the injection-appropriate lever, either sham or EKC.

The procedure for the alfentanil-trained monkeys is similar, but not identical. These animals are also trained and tested in a discrete, multiple-cycle procedure. The main difference between the alfentanil procedure and the EKC procedure is that the alfentanil monkeys are required to make 20 rather than 100 responses, and they receive a single pellet for correct responses. They can receive as many as 10 pellets during the 5-min, food-availability period of each cycle, but each pellet is delivered after 20 responses. Because in this procedure, monkeys can switch from one lever to another following the delivery of food, an additional criterion is added for satisfactory performance. In addition to making 90% or more of their responses on the correct lever, the monkeys must make fewer than 20 responses on the incorrect lever prior to delivery of the first food pellet of each cycle. Tests of the discriminative stimulus effects of submitted drugs in the alfentanil-trained monkeys are also done using a cumulative dosing procedure with dosing criteria identical to those used in the EKC-trained monkeys.

The procedure for studying discriminative stimulus effects in morphine-treated monkeys has been described previously (France and Woods, 1989). Daily sessions are comprised of a 10-min time out during which lever presses have no programmed consequence and a 5-min response period during which green stimulus lights are illuminated and signal the activation of a schedule of stimulus-shock termination. Sessions consist of between two and six discrete, 15-min cycles with each cycle. Under these experimental conditions electric shock is scheduled to be delivered to the subject's feet every 15 seconds; monkeys can terminate the lights and postpone scheduled shocks for 30 seconds by pressing

five times consecutively (*i.e.*, fixed-ratio 5) the lever appropriate for the solution administered during the first minute of the time out (left lever, saline; right lever, naltrexone). Monkeys receive an injection of saline (0.1 ml/kg) or drug (0.01 mg/kg naltrexone) during the first minute of each time out. On drug training days a single injection of naltrexone is administered during one time out and for that cycle and all subsequent cycles on that day only responding on the right lever postpones shocks. A variable number of saline cycles (0-5) precede the naltrexone cycle and on some days saline is administered during the time out of all cycles. Under these conditions monkeys switch their response choice from the saline lever to the naltrexone lever with complete generalization occurring in all three subjects at a dose of 0.01 mg/kg. Responding on the naltrexone lever is accompanied by other behavioral effects indicative of opioid withdrawal (*e.g.*, irritability, miosis, salivation). Moreover, when saline is substituted for the daily injection of 3.2 mg/kg of morphine monkeys respond predominantly on the naltrexone lever and show directly observable signs of withdrawal; the discriminative stimulus and other effects produced by morphine abstinence are reversed by some opioid agonists (*e.g.*, alfentanil; France and Woods, 1989; France et al., 1990).

For test sessions increasing doses of drug are administered during the first minute of consecutive time outs and five consecutive responses on either lever postpone shocks. In monkeys that receive 3.2 mg/kg of morphine 3 hours earlier, increasing doses of a test compound are administered up to doses that produce an average of at least 80% responding on the naltrexone lever or to doses that disrupt responding and result in the delivery of electric shock. Drugs that do not substitute for naltrexone (*i.e.*, precipitate withdrawal) are also studied for their ability to reverse responding on the naltrexone lever in morphine-abstinent (*i.e.*, withdrawn) subjects. Test compounds are studied using a cumulative-dosing procedure in morphine-abstinent monkeys up to doses that reverse completely responding on the naltrexone lever (<20%) or to doses that disrupt responding. Some compounds that substitute for naltrexone also are studied for their capacity to prevent the effects of cumulative doses of opioid agonists. Monkeys that receive saline three hours earlier, rather than the daily injection of morphine, receive saline (control) or a single injection of test compound during the first cycle and increasing doses of agonist (alfentanil or morphine) during subsequent cycles. Agonists are administered up to doses that produce a switch from the naltrexone lever to the saline lever or to doses that disrupt responding and result in the delivery of electric shock.

THERMAL ANALGESIA IN RHESUS MONKEYS

The tail withdrawal procedure used to study analgesic effects of test compounds in rhesus monkeys has been described previously (Dykstra and Woods, 1986). Monkeys are restrained loosely at the neck and arms while seated in Plexiglas primate chairs. For tests of tail withdrawal latency, the lower 10-12 cm of the shaved tail is immersed in a thermos containing water at 40°, 50°, or 55° C and the latency until the tail is withdrawn from the thermos is recorded for each monkey at each temperature. When the tail is not withdrawn within 20 seconds (cut-off latency) the experimenter removes the thermos and a latency of 20 seconds is recorded. Experimental sessions begin with several exposures to 40°C water. Four or five monkeys are tested consecutively and the time between tail immersions for individual monkeys is 5 minutes. Generally, 40° C water does not produce tail withdrawal in rhesus monkeys (Dykstra and Woods, 1986); however, if a monkey fails to keep its tail in 40° C water for 20 seconds on at least 3 of 4 immersions, that animal is not tested further for that particular session. In a subsequent pre-test component, tails are immersed in 40°, 50°, and 55° C water. The order in which the three temperatures are presented is varied among subjects. If the latencies for tail withdrawal in the pre-test component are at or near 20 seconds for 40° C water and less than 5 seconds for 55° C water, monkeys receive the test compound. The test is identical to the pre-test, except that monkeys receive s.c. injections of drug 10 minutes prior to tail immersion. The time between immersions for individual subjects is 5 minutes or less and the order in which temperatures are presented varies among subjects and across cycles. The interinjection interval typically is 30 minutes and between four and six doses are studied in a single experiment using the cumulative dosing procedure. For some studies a single dose of an opioid antagonist is administered prior to the test compound and for other studies a single dose of test compound is administered prior to increasing doses of a μ (*e.g.*, alfentanil) or κ (*e.g.*, U-50,488) opioid agonist.

RESPIRATORY STUDIES IN RHESUS MONKEYS

The effects of test compounds on ventilatory function are studied in rhesus monkeys breathing air or 5% CO_2 in air (France and Woods, 1990; Howell et al., 1988). Monkeys are restrained at the neck and waist while seated in a Plexiglas primate chair. Normal air or 5% CO_2 in air is delivered at a rate of 10 1/min into a sealed helmet placed over the subject's head. Changes in pressure within the helmet are measured and recorded by a transducer and a microprocessor, and are transformed according to known standards to frequency of respiration (f) in breaths/minute and to tidal volume (V_T) in ml/inspiration. Data are recorded continuously during 23-minute exposures to air alternating with 7-minute exposures to CO_2. The last 3 minutes of exposure to CO_2 are used for data analyses and are compared to the last 3 minutes of exposure to air only. Increasing doses of drug are administered during the first minute of consecutive time outs so that the interinjection interval is 30 minutes. For some studies a single injection of an opioid antagonist is administered prior to increasing doses of a test compound and for other studies a single injection of test compound is administered prior to cumulative doses of a standard compound (*e.g.*, alfentanil).

SELF-ADMINISTRATION BY MONKEYS

Tests of self-administration determine the ability of the drug to maintain responding in monkeys trained to self-inject codeine. Each of at least three monkeys is studied with saline as a negative control and a number of doses of the test compound until a maximum rate of responding was obtained or until, in the absence of evidence of a reinforcing effect, observable changes in behavior are produced by the compound.

The schedule of intravenous drug delivery is a fixed-ratio 30; when a light above a lever is illuminated, the 30th response produce an intravenous drug injection accompanied by another light that is illuminated during drug delivery. After each injection, a 45 sec timeout period occurs. A component of the session ends after 20 injections have been received or 25 min have passed, whichever occurs first. Different doses of the drug are available during each of four components of a session. Other procedural details are given in Winger *et al.* (1989).

***

AFFILIATION

The Drug Abuse Basic Research Program, Departments of Pharmacology and Psychology, University of Michigan, Ann Arbor, MI 48109-0632.

DEPENDENCE STUDIES OF NEW COMPOUNDS IN THE RHESUS MONKEY, RAT AND MOUSE (2000)

M. D. Aceto, E. R. Bowman, L. S. Harris and E. L. May

Department of Pharmacology and Toxicology, School of Medicine, Virginia Commonwealth University, Richmond, VA

All compounds were unknown to us when submitted by the Biological Coordinators, Dr. Arthur Jacobson, Laboratory of Medicinal Chemistry, NIDDK, NIH and Dr. Andrew Coop of University of Maryland. These studies were conducted under the auspices of the Drug Evaluation Committee in association with the College on Problems of Drug Dependence. See summary of new data in Table 1.

Dependence-Liability Studies in Rhesus Monkeys

Substitution-for-Morphine (SDS) Test. Male and female rhesus monkeys (M. mulatta) weighing 2.5-7.5 kg were used, and they received 3 mg/kg, s.c., of morphine·SO_4 every 6 hr. All the animals had received morphine for at least 3 months and were maximally dependent on morphine (Seevers and Deneau 1963). A minimal 2-week recuperation period was allowed between tests. At least 3 monkeys/dose were used. The assay (Aceto and co-workers, 1977 and 1978) was initiated by a subcutaneous injection of the test drug or control substances (morphine and vehicle) into animals in a group that had not received morphine for 14-15 hr and showed definite signs of withdrawal. Each animal was randomly chosen to receive one of the following treatments: a) a dose of the compound under investigation; b) morphine control, 3.0 mg/kg; and c) vehicle control, 1 ml/kg. The animals were scored for suppression of withdrawal signs during a 2.5-hr observation period. The observer was "blind" regarding the choice of treatments. At the end of the study, the data were grouped according to dose and drug. The mean cumulative score ± SEM was calculated and the data illustrated in figure form. If indicated, the data were analyzed using the Kruskal-Wallis Anova and post hoc Mann-Whitney U-Tests.

Precipitated-Withdrawal (PPT-W) Test. This evaluation was done under the same conditions as described above, except that the animals were administered a test compound 2-3 hr after the last dose of morphine. These animals were not in withdrawal. Naloxone·HCl (0.05 mg/kg, s.c.) served as the positive control.

Primary-Physical-Dependence (PPD) Study. Drug-naive monkeys were medicated with drug, using escalating dose regimens, periodically challenged with naloxone or placed in abrupt withdrawal. They were observed for overt behavioral signs during drug administration and when they were challenged with the antagonist, naloxone, or abruptly withdrawn from the drug.

Rat-Infusion Studies

The continuous-infusion method was reported by Teiger (1974) and certain modifications are indicated as follows. Rats were anesthetized after which each was fitted with a specially prepared cannula which was passed subcutaneously from the nape of the neck to the lateral side of the lower abdomen and then inserted into the peritoneal cavity. The cannula was anchored at both ends with silk sutures and attached to a flow-through swivel mechanism which allowed the animal to move about in the cage and eat and drink normally. The swivel was connected to a syringe which was attached to a syringe pump. The animals received 7-10 ml of solution every 24 hr.

TABLE 1 SUMMARY OF NEW DATA

NIH No.	Chemical Name or Generic Class	MOUSE					RAT		MONKEY		
		TF	TFvsM	PPQ	HP	pA_2	SM	PPD	SDS	PPT-W	PPD
10497	Normorphine	T[a]						T			
10908	4-Phenylpiperidine (Sameridine)	T[b]	T	T	T				T		
10924	Naltriben (NTB)			T[c]							
10931	Endoethenomorphinan	T[d,e]		T[f]					T		
10947	γ-Hydroxybutyric Acid, Na Salt (GHBA)	T[g]		T[g]					T		
10963	(±)-6,7-Benzomorphan	T[h]	T	T	T				T		
10964	(±)-6,7-Benzomorphan (Ketocyclazocine)	T[i]	T	T	T				T	T	
10966	Carbamic Acid (Carisoprodol)	T	T	T	T				T		
10968	Dihydrohydromorphinone	T[j]	T	T	T						
10969	Tramadal·HCl (Phenyl-cyclohexanol)	T	T	T	T[k]						
10970	Phenylcyclohexanol	T	T	T	T						
10971	(+)-6,7-Benzomorphan	T	T	T	T						
10972	(-)-6,7-Benzomorphan	T[l]	T	T	T[m]						
10973	(+)-6,7-Benzomorphan	T	T	T	T				T		
10974	(-)-6,7-Benzomorphan	T[n]	T	T	T				T		
10975	Norisonicotine	T	T	T	T				T		
10976	Norisonicotine	T	T	T	T				T		
10977	Coumarin-based cyclic prodrug of DADLE	T[o]	T[o]	T[o]	T[o,p]						
10980	(-)-6,7-Benzomorphan	T[q]	T	T	T				T		
10981	(+)-6,7-Benzomorphan	T	T	T	T				T		
10982	(+)-6,7-Benzomorphan	T	T	T	T				T		

TABLE 1. SUMMARY OF NEW DATA (Continued)

10983	(-)-6,7-Benzomorphan	T	T	T	T				T		
10984	N-Methylmorphinan	T[r]	T	T	T				T		
10991	2-Chloropyridine	T			T[s]						
10993	(-)-6,7-Benzomorphan	T	T	T	T				T		
10998	Heterocodeine·HCl	T[u]	T	T	T				T		
10999	(-)-6,7-Benzomorphan	T	T	T	T				T		
11000	(+)-6,7-Benzomorphan	T	T	T	T				T		
11001	4-Phenylpiperidine	T	T	T	T				T		
11002	4-Phjenylpiperidine	T	T	T	T				T		
11008	S-(+)-Mecamylamine·HCl	T			T						
11009	R-(-)-Mecamylamine·HCl	T			T						
11010	(±)-Mecamylamine·HCl	T			T						

T = Test Performed

[a]Special: Naltrindole, nor-BNI, β-FNA and naloxone vs ED80 of NIH 10497 in TF. [b]Special: Naloxone vs ED80 of NIH 10908 in TF. [c]Special: Nor-BNI and β-FNA vs ED80 of NIH 10924 in TF. [d]Special: Naloxone vs ED80 of NIH 10931 in TF; NIH 10931 vs ED80 of DPDPE in TF. [e]Special: Time-course studies in TF. [f]Special: Naltrindole vs ED80 of NIH 10931 in PPQ. [g]Special: Oral and intravenous studies in TF and PPQ. [h]Special: Naloxone, β-FNA, nor-BNI and naltrindole vs ED80 vs ED80 of NIH 10963 in TF. [i]Special: Naloxone, β-FNA, nor-BNI and naltrindole vs ED80 vs ED80 of NIH 10964 in TF. [j]Special: Naloxone, β-FNA, nor-BNI and naltrindole vs ED80 vs ED80 of NIH 10968 in TF. [k]Special: Naltrindole vs ED80 of NIH 10969 in PPQ. [l]Special; Naltrindole vs ED80 of NIH 10972 in PPQ. [m]Special: β-FNA, nor-BNI, ED80 of U-69,593 and DPDPE vs ED80 of NIH 10972 in TF. nSpecial: Naloxone, nor-BNI, naltrindole and β-FNA vs ED80 of NIH 10974 in TF. [o]Special: Two different samples were tested. [p]Special: Naltrindole vs ED80 of NIH 10977 in PPQ. [q]Special: Naloxone, nor-BNI, naltrindole and β-FNA vs ED80 of NIH 10980 in TF. [r]Special: Nor-BNI, naltrindole and β-FNA vs ED80 of NIH 10984 in TF; special time-course study of antagonist effects of NIH 10984 vs morphine. [s]Special: b-FNA (i.c.v.) vs ED80 of 10991 in PPQ; nor-BNI, naltrindole and mecamylamine vs ED80 of NIH 10991 in PPQ. [u]Special: Naloxone, b-FNA (i.c.v.),nor-BNI and naltrindole vs ED80 of NIH 10998 in TF.

Substitution-for-Morphine (SM) Test. The rats received morphine·SO_4 (50 mg/kg/24 hr on the first day, 100 mg/kg/24 hr on the second day, and 200 mg/kg/24 hr from days 3 and 4). Then, a test drug was substituted for 2 days. The morphine controls received an infusion of sterile water for injection. The animals were observed for changes in body weight and for behavioral-withdrawal signs for 0.5 hr at 6, 24, 48, 72 and/or 96 hr after stopping the infusion of morphine.

Primary-Physical-Dependence (PPD) Study. The rats received test compound, as specified above, for 4-6 days and then, were placed in abrupt withdrawal and observed for overt behavioral signs.

Mouse-Antinociception Tests

Male mice, weighing 20-30 g, were used. All drugs were dissolved in distilled water or in the vehicle indicated and injected subcutaneously (s.c.). At least three doses were tested, and 6-10 animals per dose were used. When applicable, ED50's were calculated by using computerized probit analysis. The results obtained with reference compounds are summarized in Table 2. Occasionally, when requested, drugs were given orally (p.o.) or intravenously (i.v.) and the pretreatment times are indicated in the text.

Tail-Flick (TF) and (TF vs M) Assays. The procedure and modifications were described (D'Amour and Smith, 1941 and Dewey et al., 1970 and 1971) in the literature. Briefly, the mouse's tail was placed in a groove which contained a slit under which was located a photoelectric cell. When the heat source of noxious stimulus was turned on, the heat focused on the tail, and the animal responded by flicking its tail out of the groove. Thus, light passed though the slit and activated the photocell which, in turn, stopped the recording timer. The heat source was adjusted to produce tail flick of 2-4 sec under control conditions. Mice were injected with drug or vehicle and tested 20 min later. In three assays for antagonism of the antinociceptive effect, the potential antagonists were administered 10 min before the agonist, and evaluation occurred 20 min later.

Phenylquinone Abdominal-Stretching (PPQ) Assay. The procedure was reported previously (Pearl and Harris, 1966). The mice were injected with test drug and 10 min later received 2.0 mg/kg intraperitoneally (i.p.) of a freshly prepared paraphenylquinone (PPQ) solution. The mice were then placed in cages in groups of two each. Ten min after the PPQ injection, the total number of stretches per group were counted over a 1-min period. A stretch was characterized by an elongation of the mouse's body, development of tension in the abdominal muscles, and extension of the forelimbs. The antinociceptive response was expressed as the percent inhibition of the PPQ-induced stretching response.

Hot-Plate (HP) Assay. The method was also reported previously (Eddy and Leimbach, 1953 and Atwell and Jacobson, 1978). The hot plate was held at 55°C. Mice were placed on the hot plate and activity was scored if the animal jumped or licked its paws after a delay of 5 sec or more, but no more than 30 sec beyond the control time.

Table 2

Comparative Data (ED50, mg/kg s.c.) [95% C.L.] of Selected Standards in 4 Mouse Agonist-Antagonist Tests

Drug	Tail-Flick	Tail-Flick Antagonist	Phenylquinone	Hot-Plate
Pentazocine	15% at 10.0	18 (12-26)	1.7 (1.0-2.5)	13% at 30.0
Cyclazocine	17% at 1.0[a]	0.03 (0.02-0.78)	0.01 (0.005-0.03)	25% at 9.0
Nalorphine·HCl	None at 10.0	2.6 (0.7-1.0)	0.6 (0.03-1.44)	13% at 30.0
Naloxone·HCl	None at 10.0	0.04 (0.0-0.09)	No Activity	- - - -
Naltrexone·HCl	None at 10.0	0.007 (.002-0.02)	No Activity	- - - -
Morphine·S04[b]	1.92 (0.89-4.14)	Inactive	0.4[b] (0.2-0.8)	0.85 (0.39-1.86)
Codeine·P04	- - - -	Inactive	8.25 (5.12-13.29)	6.4 (2.4-16.8)
Meperidine·HCl	8.37 (4.59-15.27)	Inactive	- - - -	4.6 (1.18-11.7)

[a]Mice were ataxic at 3.0 and 10.0 mg/kg but there was no further increase in reaction time

[b]ICR - Harlan-Sprague-Dawley Inc.

Calculation of Apparent pA_2. Using the tail-flick or PPQ assay, the apparent pA_2 and 95% confidence limits were calculated using Schild and constrained plots as described in Tallarida and Murray (Manual of Pharmacologic Calculations with Computer Programs, 2nd ed., Springer Verlag, NY., 1987).

Briefly, mice were pretreated with vehicle or various doses of antagonist followed 10 min later by an injection of agonist. The mice were tested 30 min after receiving the antagonist. Dose-response lines for antinociception were plotted using at least 3 doses of each opioid agonist in the presence of vehicle or one of the selected doses of antagonist. ED5Os were estimated according to the method of Litchfield and Wilcoxon (J. Pharmacol. Exp. Ther., 96, 399, 1949). Each dose ratio (x) was calculated by dividing the ED50 of the opioid in the presence of a given dose of antagonist by that of the agonist alone. Log (x-l) was plotted against the negative logarithm of the molar dose of the antagonist. At least 3 logs (x-l) were plotted. The pA_2 values for the antagonists were calculated from the point of intersection of the regression line with the abscissa. See Table 3 for summary of results.

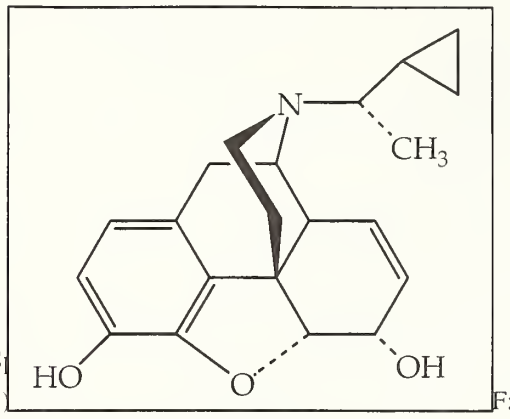
Table 3. Apparent pA_2 values[a] using the mouse tail-flick assay

Treatment Antagonist/Agonist	Schild Plot pA_2 (95% C.L.) Slope	Constrained Plot pA_2 (95% C.L.)
1) Naloxone/Morphine	7.2 (7.0-7.4)-1.2	7.3 (7.1 - 7.6)
2) Naloxone/Sufentanil	7.0 (6.5 - 7.5)-1.0	7.0 (6.8 - 7.1)
3) Naloxone/Mirfentanil	7.6 (7.3 - 8.0)-0.7	7.2 (6.9 - 7.5)
4) Naloxone/NIH 10672 (Enadoline) (selective kappa agonist)	6.1 (5.6 - 6.6)-1.2	6.6 (6.3 - 7.0)
5) Naloxone/U-50,488 (kappa agonist)	6.6 (6.3 - 6.9)-1.1	6.2 (5.9 - 7.3)
6) Naloxone/(-)-Nicotine	5.3 (5.3-5.3)-0.5	-
7) Nalmefene/Morphine	8.0 (7.6 - 8.3)-1.1	8.0 (7.7 - 7.6)
8) Naltrexone/Morphine	7.7 (4.9 - 10.5)-0.8	7.6 (7.1 - 8.3)
9) (-)-Quadazocine/Morphine	6.8 (6.7 - 7.0)-0.9	6.8 (6.1 - 7.6)
10) (-)-Quadazocine/Enadoline	6.2 (6.1 - 6.2)-1.7	6.7 (6.6 - 6.8)
11) nor BNI/Enadoline	6.5 (5.9 - 7.0)-1.3	6.6 (5.9 - 7.3)
12) Mecamylamine/(-)-Nicotine	6.6 (6.2 - 6.9)-0.9	-

[a]Negative logarithm of the molar concentrations of antagonist required to produce a two-fold shift of the agonist dose-response curve to the right. Competitive antagonism can be assumed when slope = -1. pA_2 provides a measure of the relative potency and affinity of the antagonist. When the slope differs significantly from unity, this may indicate non-equilibrium conditions, interactions with multireceptors, receptor sensitization, precoupling mechanisms, or multiple drug properties. With a constrained plot, the slope of the regression line is restricted to slope of - 1.

Special Intracerebroventricular Tail-Flick and PPQ Assays. In order to develop an in-vivo agonist and antagonist model to correlate with the in-vitro binding data of the various opioid receptor types (mu, kappa and delta), we chose the mouse Tail-Flick and PPQ tests and a variety of routes of administration. The intracerebroventricular (i.c.v.) route was chosen to accommodate thee fact that no delta agonist is available which is active by peripheral routes of administration

NIH 10497 N-(1*R*)-1-Cyclopropyl)ethylnormorphine hydrochloride



MOUSE DATA - ED50 OR AD50
(95 % C.L.) (mg/kg or % change)

1) TF – 2.0 (0.6 – 6.6)[a]
2) TF vs. M – Inactive at 1, 10 and 30[a]
3) PPQ – 0.03 (0.01 – 0.2)[a]
4) HP – NT

[a]Reported in NIDA Res. Monog. 95. 1989

S
1 F: 2.98 (1.19 – 7.48)

2) Opioid subtype tests
a) β-FNA (i.c.v.) vs ED80 of NIH 10497 (s.c.) in TF: Inactive at 1, 10 and 30 μ/brain.
b) nor-BNI (s.c.) vs ED80 of NIH 10497 (s.c.) in TF: AD50 = 11.17 (2.9 -42.9)
c) Naltrindole (s.c.) vs ED80 of NIH 10497 (s.c.) in TF: 10% at 1, 0% at and 23% at 30.

MONKEY DATA (Reported in NIDA Monog. 95, 1989)
(SDS)

NIH 10497 substituted completely for morphine. The drug acted promptly and its duration of action was about 2 hr (see Fig NIH 10497-SDS). In addition, this drug is slightly less potent than morphine. Many drug-related side effects were seen including body sag, jaw sag, slowing, staring, and salivation. The incidence of drowsiness was more than observed in morphine-treated controls. [In this context, salivation suggested kappa agonist activity].

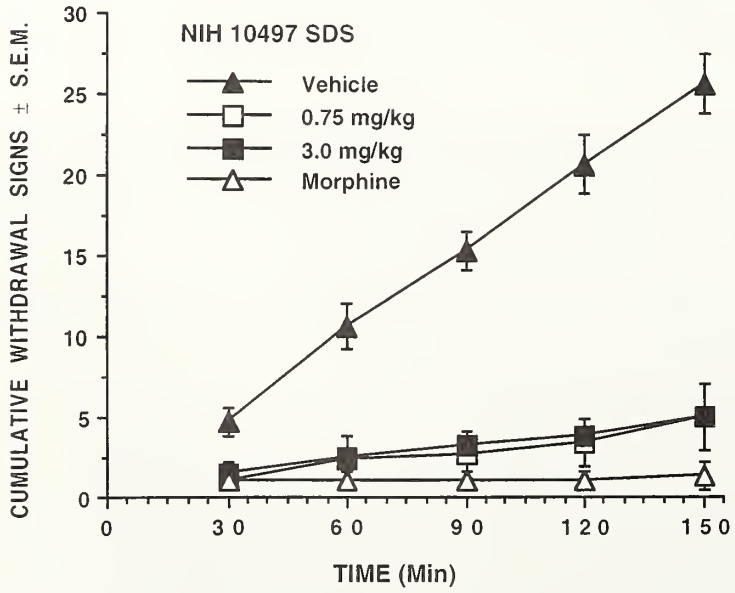


Fig NIH 10497–SDS. Results of study in which single doses of NIH 10497 were substituted for morphine in morphine-dependent monkeys in withdrawal.

NIH 10497 (Continued)

Comment: Apparently, NIH 10497 is a selective kappa agonist in both species. Some weak delta-opioid-receptor agonist activity was observed.

RAT CONTINUOUS INFUSION ASSAY (PPD) - New Study

Primary physical dependence study

Each rat was randomly allocated a treatment regimen. They were then assigned to a cage on a rack. The 6-day morphine dose regimen that was used by Teiger (1974) was modified by us and shortened to 4 days because studies in our laboratory indicated that the withdrawal syndromes were qualitatively and quantitatively similar. The dose regimen for morphine was 50 mg/kg day on the first day, 100 mg/kg day on day 2, and 200 mg/kg/day on days 3 and 4. NIH 10497's low-dose regimen was the same as for morphine. The high-dose regimen was double that of the low-dose regimen.

Physiological and Behavioral Measurements

During the infusion of vehicle, NIH 10497, or morphine, the rats were weighed and observed daily for 1 hr for overt behavioral signs. Body weight was recorded daily. The sign wet-dog shakes was quantified. Irritability was scored as proposed by Teiger (1974). Scoring for this sign was as follows: 0 (remains tame when touched and on being grasped and lifted); 1 (remains tame when touched and on being grasped and lifted makes only a feeble attempt to wiggle free); 2 (remains tame when touched but when grasped and lifted claws, bites and or vocalizes); and, 3 (reacts to initial touch by vocalizing and biting and attempts to grasp it by rolling over on its back and clawing). All other behavioral signs were simply noted. A trained observer was blind regarding treatment assignments.

Statistical Analysis

The data from above were combined and analyzed. Quantified data were assessed using repeated measures ANOVA. One factor ANOVA was used to evaluate daily blocks of data. If overall significance was found, Fisher's LSD test was used for post-hoc comparisons. Scored data were analyzed using the nonparametric Kruskal-Wallis one-way ANOVA. Post hoc comparisons of nonparametric data were made using the Mann-Whitney U test. In all cases significance was set at the 95% level. The StatView statistical package (Brainpower, Inc., Agoura Hills, CA) was utilized for these analyses.

Drugs and Solutions

NIH I0497 was forwarded to us by Dr. A. Jacobson of NIH. Morphine sulfate was purchased from Mallinckrodt, Inc., St. Louis, MO. All drugs were dissolved in distilled water and solutions were prepared daily.

Results

Body Weight Loss - These results are displayed in Fig. 1 (Rat PPD-Body Weight). Two-factor repeated measures analysis of variance revealed significant differences among treatment groups ($F = 5.33$, $P = 0.0083$) and days ($F = 21.048$, $P = 0.001$). One factor analysis of body weights at the start of the experiment indicated no significant differences ($F = 0.172$, $P = 0.9142$). One factor ANOVAs for day 3($F = 3.718$, $P = 0.0306$) , day 4 ($F = 11.607$, $P = 0.0002$), day 5 ($F = 7.882$, $P = 0.0014$), day 6 ($F = 19.747$, $P = 0.0001$) day 7 ($F = 4.1$, $P = 0.0221$), and day 8 ($F = 25.349$, $P = 0.0001$) but not days 1 ($F = 1.899$, $P = 0.1959$), and 2 ($F = 2.175$, $P = 0.1263$) showed significant differences among treatments.

During the infusion of morphine, the rats initially showed small increases in body weight during the first 2 days when compared to that of the vehicle controls; the gain was statistically significant on day 1. After morphine was abruptly withdrawn and vehicle substituted, there was a precipitous and significant loss of body weight during the first 24 hr (day 5) followed forty-eight hr later (day 6), by an even greater loss. Although body weights appeared to be recovering during the rest of the experiment, weight loss was still significantly reduced compared to that of vehicle controls.

NIH 10497 (Continued)

In sharp contrast with the results obtained with the morphine controls, body weight decreased in a dose-dependent manner in the rats treated with NIH 10497 during its administration and began recovering within 24 hr after it was abruptly discontinued. For the high-dose regimen of NIH 10497, weight loss was significant compared to the vehicle control group beginning on day 4. It is interesting that although weight loss was still significant compared to the vehicle group at the end of the experiment it was also significantly less than that of the morphine-treated group.

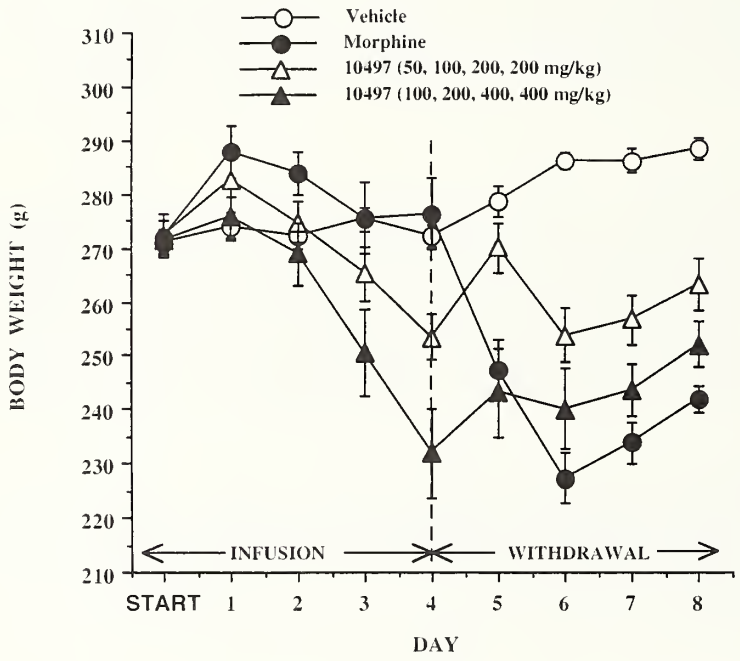


Fig.1. Rat PPD: Body Weight

Wet-dog Shakes - The results are depicted in Fig. 2 (Rat PPD-Wet-Dog Shakes). When examined for overall differences, 2-factor repeated measures analysis of variance indicated that differences among treatments were significant only for days ($F = 5.217$, $P = 0.001$).

One factor ANOVAs for day 6 (48 hr post withdrawal) indicated a significant difference among treatments ($F = 3$, $P = 0.0578$). Comparison of the results of the morphine-treated group with those of the vehicle group was the only comparison among groups that was significant.

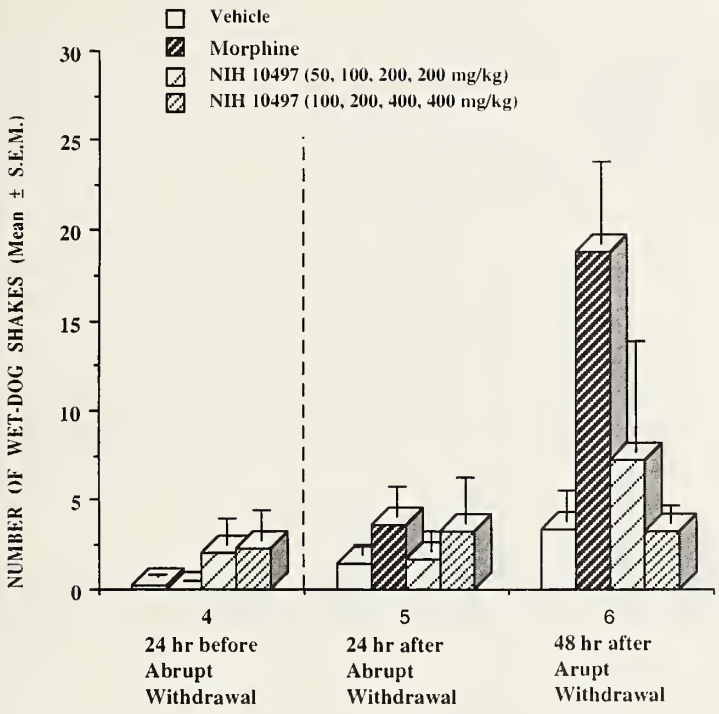


Fig. 2. Rat PPD: Wet -Dog Shakes

Irritability - Fig. 3 (Rat PPD-Irritability) displays the results obtained with this sign. The critical value (based on 4 treatments and 3 degrees of freedom) for this experiment using Kruskal-Wallis ANOVA is $X^2_{0.05}$ (3) = 7.82 and H for day 5 was 0.357. Post hoc comparisons (Mann-Whitney one-tail test) on this day indicated that only the results of the morphine-treated group showed a significant difference when compared to those of the vehicle group.

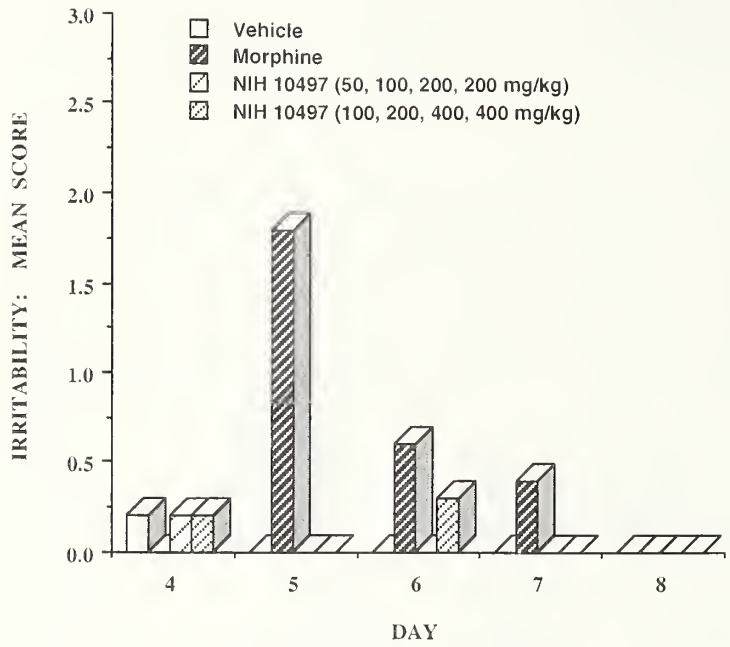
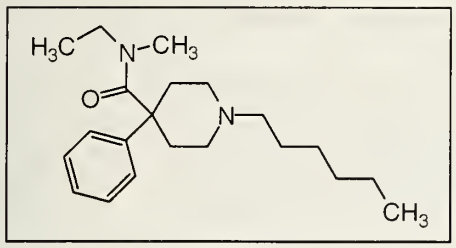


Fig. 3. Rat PPD: Irritability

At dose regimens approximately equal to and double those of morphine sulfate, NIH 10497 did not produce body-weight loss, the most reliable index of physical dependence on morphine (Aceto 1990). Neither did it increase the degree of irritability in response to handling, another significant mu-opioid receptor agonist abstinence sign (Himmelsbach *et al.*, 1935 and Aceto, 1990). Finally, it did not express another important morphine-like abstinence sign designated wet-dog shakes. These results suggest that NIH 10497 is relatively free of mu-opioid induced physical dependence liability.

Comment: NIH 10497 has a novel profile of activity. It lacks mu-opioid receptor properties and has selective kappa- and weak delta-opioid receptor effects. Since it substituted for morphine in morphine-dependent monkeys and appears to be free of mu-opioid physical-dependence liability, it may prove to be useful in the pharmacotherapy of heroin-like abuse. However, species difference may have accounted for these results.

NIH 10908 Sameridine hydrochloride
(N-Ethyl-N-methyl-1-hexyl-4-phenyl-4-piperidinecarboxamide hydrochloride)



MOUSE DATA - ED50 OR AD50
(95 % C.L.) (mg/kg or % change)

1) TF - 7.4 (4.1 - 13.3)[a]
2) TF vs M - Inactive at 1, 10 and 30[a]
3) PPQ - 2.5 (1.0 - 5.9)
4) HP - 8.8 (5.2 - 15.0)[b]

[a]One of 6 died at 20
[b]Three of 8 died at 30

Special Test: Naloxone AD_{50} vs ED_{80} of NIH 10908 in TF = 0.07 (0.03 - 0.2)

MONKEY DATA
(SDS)

NIH 10908 dose-dependently substituted for morphine at doses of 1 and 4 mg/kg. However, at the high dose the signs designated scratching, jaw and body sag, ataxia and slowing were observed (see Fig NIH 10908-SDS).

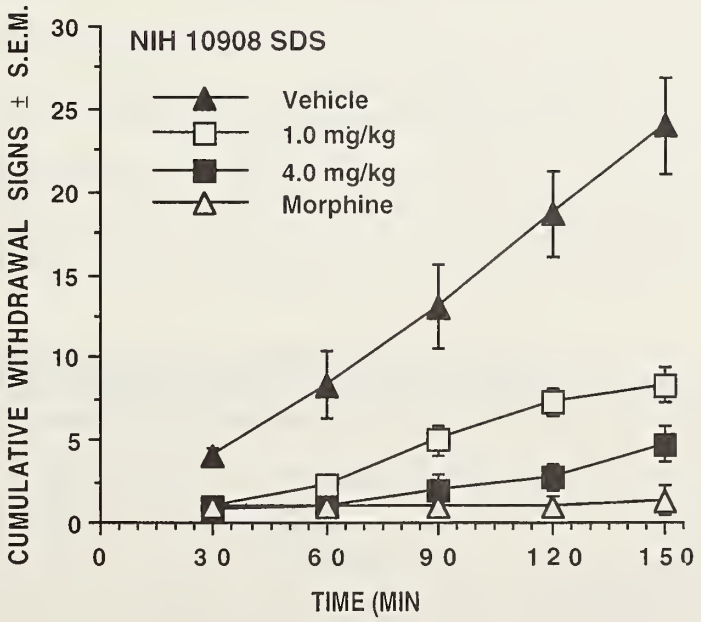
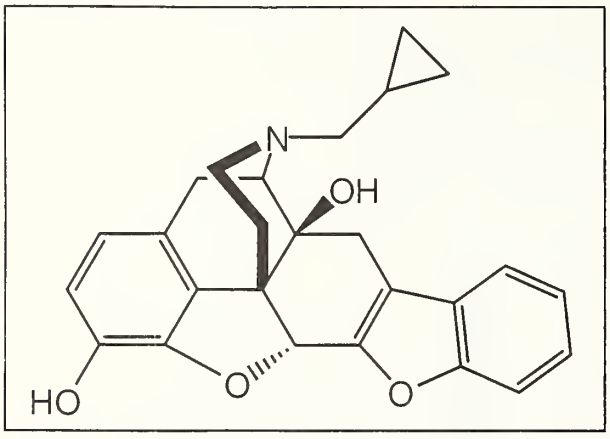


Fig NIH 10908-SDS. Results of study in which single doses of NIH 10908 were substituted for morphine in morphine-dependent monkeys in withdrawal.

Comment: The profile of biological activity suggests opioid properties for this compound. Specificity for the mu-opioid receptor is indicated by the special naloxone-antagonism test. In addition, NIH 10908 seems unusually lethal to mice.

NIH 10924 17-Cyclopropylmethyl-6,7-dehydro-4,5β-epoxy-3,14-dihydroxy-6,7-2',3'-benzofuranomorphinan methanesulfonate (Naltriben methanesulfonate; NTB methanesulfonate)



MOUSE DATA - ED50 OR AD50
(95% C.L.) (mg/kg or % change)

1) TF - Inactive at 1, 10 and 30[a,b,c,d]
2) TF vs M - 0.99 (0.42 - 2.35)[a,d]
3) PPQ - 4.2 (3.1 - 5.7)[a,d]
4) HP - Inactive at 1 and 3, 25% at 30[a,d]

[a]10% DMSO aqueous solution.
[b]1 of 6 had convulsions and died at 10 and 6 of 6 had convulsions and died at 30.

[c]Naltrindole pretreatment did not abolish lethal effects at 30.
[d]Published Previously, see NIDA Monog. 179, 354, 1998.

Opioid Subtype Tests:

Previously Published Data (NIDA Monog. 179, 354, 1998)
Special: NIH 10924 (s.c.) vs ED80 DPDPE (i.c.v.) in TF: AD50 = 3.15 (1.36 - 7.27) mg/kg.
Special: Naltrindole (s.c.) vs NIH 10924 ED80 in PPQ: Inactive at 1, 10 and 30.

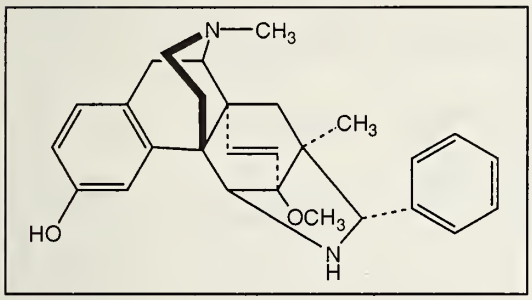
New Data
Special: Nor-BNI (s.c.) vs NIH 10924 ED80 (s.c.) in PPQ: Inactive at 1 and 10 and 30.
Special: β-FNA (i.c.v.) vs NIH 10924 ED80 (s.c.) in PPQ: Inactive at 1, 3, 10 and 30.

MONKEY DATA
(SDS)

Not tested.

Comment: NIH 10924 has a curious mix of agonist/antagonist effects. The three opioid subtype antagonists are inactive vs the ED80 of this compound in the PPQ test. However, it does have delta antagonist activity in the tail-flick test. Because naltrindole did not block the convulsions, they are probably not delta-opioid related.

NIH 10931 N-Methyl[5β,7β,3',5']pyrrolidino-2'-[*S*]-phenyl, 7α-methyl, 3-hydroxy, 6-methoxy- 6,14 endoethenomorphinan dihydrochloride



MOUSE DATA - ED50 OR AD50
(95 % C.L.) (mg/kg s.c., µg/brain (i.c.v.) or % change)
1) TF - 0.005 (0.003 - 0.008)[a,b]
TF - 0.07 (0.03 – 0.15) µg/brain[c]
2) TF vs. M - Inactive at 1, 10 and 30[a,b]
3) PPQ - 0.004 (0.002 - 0.006)[a,b]
4) HP - 0.01 (0.003 - 0.29)[a,b]
[a]Reported 1/9/98
[b]Pretreatment time is 10 min before morphine

Naltrindole pretreatment did not abolish lethal effects at 30.

Published Previously, see NIDA Monog. 179, 354, 1998

New Data

Special Testss:

1) Naloxone vs ED80 of NIH 10931 in TF: AD50 = 0.02 (0.01 - 0.04) (See [a,b] above)

2) Opioid Subtype Tests:
a) NIH 10924 (s.c.) vs ED80 DPDPE (i.c.v.) in TF: AD50 = 3.15 (1.36 - 7.27) mg/kg.
Naltrindole (s.c.) vs NIH 10924 ED80 in PPQ: Inactive at 1, 10 and 30.

3) ED80 Agonist Time-Course Study: These results are depicted in Fig NIH 10931-Time-Course Study below.
At this dose (ED80), antinociceptive activity lasted at least 8 hr.

4) Subcutaneous Antagonist Time-Course Study: The data are reported in the Table 1 (Antagonist activity of NIH 10931) below. Apparently, antagonist activity is not evident until 24 hr have elapsed, peaks at 72 hr and is still present at 168 hr (7 days). Also, note that 30 mg/kg during the time periods 4 and 24 hr, no antagonist activity was present, suggesting that at this high dose, agonist effects predominated. (See Table 1).

5) Intracerebroventricular Time-Course Study.
Some opioid agonist and delayed opioid antagonist activity now shown in Table 2 (Effects (i.c.v.) pretreatment of NIH 10931 in the TF test).

Table 1. Antagonist activity of NIH 10931 vs the ED80 of morphine sulfate in the tailflick test.

Pretreatment Time (hr)	**AD50 (mg/kg s.c.)[a]**	**% Antagonism (mg/kg s.c.)**
4	-	0% antagonism at at 1, 10 and 30
24	-	26% at 1, 51% at 10 and 0% at 30
48	2.9 (1.0 - 8.4)	-
72	1.4 (0.5 - 4.3)	-
96	3.6 (0.8 - 10.7)	-
120	-	64% at 1, 83% at 10 and 52% at 30
144	-	51% at 1, 2% at 3, 45% at 10 and 69% at 30
168	-	0% at 1, 0% at 3, 61% at 10 and 30% at 30

[a]The variability of the data may be related to the fact that only 6 mice per dose regimen were tested and that the expression of antagonist and antagonist effects was related to the dose. For example, at 30 mg/kg during the time periods 4 and 24 hr, no antagonist activity was observed. In fact, at this dose the mice exhibited Straub tails.

Table 2. Effect of (i.c.v.) NIH 10931-pretreatment time on morphine-induced antinociception (ED80, s.c.) in the tail-flick test.

Pretreatment Time	AD50 or % Antagonist Effect ug/brain	Comment
10 min	Inactive at 1, 10 and 30.	Loss of righting reflex at 10 and 30 ug/brain.
8hr	AD50 = 10.67 (2.9-39.1).	Straub tail at 30 ug/brain observed before morphine.
24 hr	21% at 1, 37% at 3, 71% at 10 and 18% at 30.	1/6 died at 30 ug/brain before morphine could be given.

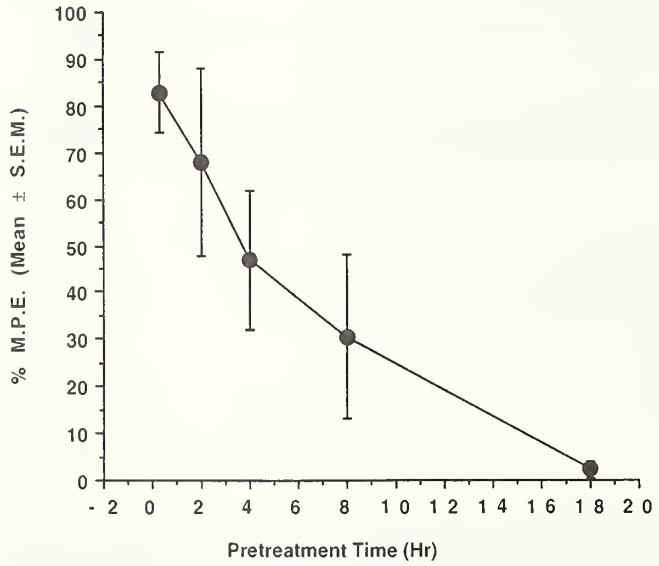


Fig. NIH 10931 (Time-course study) NIH 10931-induced antinociception in mice (ED80 dose 0.5 mg/kg s.c.).

MONKEY DATA
(SDS)

NIH 10931 substituted for morphine in a dose-related manner (see fig NIH 10931-SDS). Onset was prompt and duration of action was at least 2 1/2 hr. Potency is conservatively estimated to be 500 to 2000 times that of morphine. Some eyelid ptosis and body sag were noted at the higher dose.

NIH 10931 (Continued)

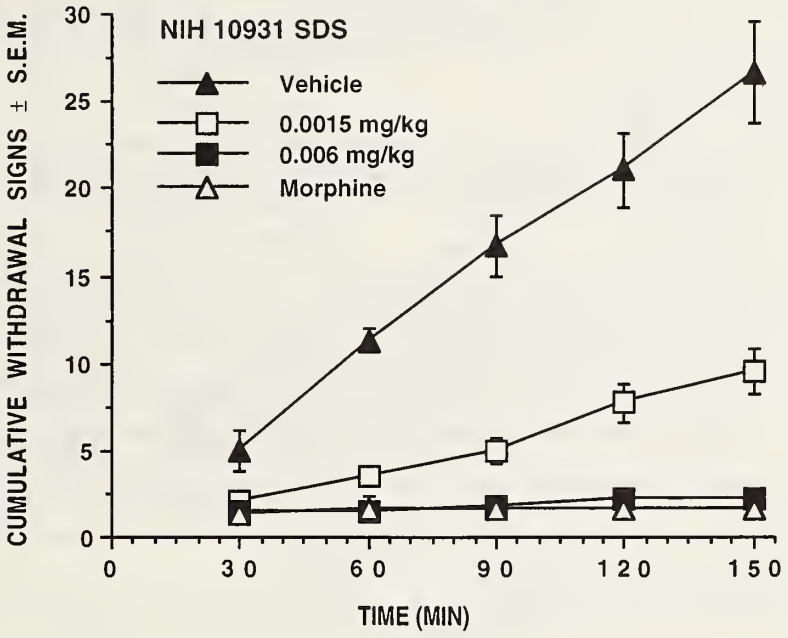
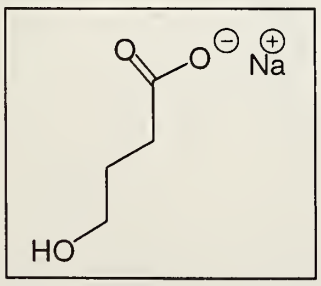


Fig NIH 10931-SDS. Results of study in which single doses of NIH 10931 were substituted for morphine in morphine-dependent monkeys in withdrawal.

Comment: In the mouse, when given by the subcutaneous route, this compound displayed potent opioid antinociceptive effects of long duration followed by antagonist activity lasting at least 168 hr. When given by the intracerebroventricular route, it followed the same pattern of activity as in the subcutaneous study except that the duration of action was abbreviated. NIH 10931 is a potent long-acting mu-opioid agonist which, possibly, is metabolized to an (irreversible ?) opioid antagonist with a very long duration of action. In the monkey, NIH 10931 behaved essentially as a potent mu agonist.

NIH 10947 Gamma-Hydroxybutyric Acid (Na Salt)



Gamma-Hydroxybutyric Acid (GHBA), a precursor and metabolite of gamma-aminobutyric acid which has been used in Europe, as a general anesthetic and hypnotic, as an aid in childbirth, in the treatment of alcoholism and in anxiety attendant with detoxication from cocaine and amphetamines, depression and other conditions, has also gained popularity as a fashionable recreational drug. Because little is known about its interaction with opioids, this

study was initiated. GHBA, per se, (at 30, 60, 80, and 120 mg/kg s.c.) had little effect on the normal reaction time in the tail-flick test. When these doses of GHBA were coadministered with the ED25 of morphine, dose-related synergism was observed. In mice made completely tolerant to morphine antinociceptively (25 mg/kg.s.c., 4 times a day for 4 days), GHBA (60 mg/kg s.c.) in combination with morphine partially restored antinociception. Naloxone (1 mg/kg s.c.) nearly abolished this effect.

Table Antinociceptive Effects of GHB in the Mouse Tail Flick and Paraphenylquinone Assays[a]

Test	Route of Administration	Pretreatment Time	ED50 or AD50 (95% C.L.) (mg/kg or % Change)
Tailflick	i.v.	20 min	Inactive at 60 and 52% at 120
"	s.c.	20 min	Inactive at 1, 10, 30 and 60
"	p.o.	20 min	Inactive at 60 and 51% at 120
"	p.o.	1 hr	Inactive at 60 and 120
PPQ	i.v.	20 min	30.88 (15.34 - 62.17)

[a]Reported in NIDA Monograph 179, p 363, 1999.

MONKEY DATA

(SDS)

The results suggest an inverse dose-response relationship (see Fig NIH 10947-SDS) for GHBA regarding attenuation of withdrawal signs in withdrawn morphine-dependent monkeys. Statistical analysis of the data obtained at 150 min, revealed by Kruskal-Wallis one-way analysis of variance, predicted highly significant differences among treatment regimens ($H=23.26\_\chi^2\_0.005 = 16.75$). The Mann-Whitney U test was used to assess between treatment comparisons. The results indicated that all treatment regimens except the GHBA 120 mg/kg and GHBA 240 mg/kg were significantly different from vehicle (at U = 6 or less, P = 0.05 or less). In addition, all GHBA-treated group withdrawal scores were significantly higher than those in morphine-treated monkeys (U= 6 or less, P = 0.01 or less). Finally, The scores of the low-dose GHBA group (7.5 mg/kg) were significantly less than the scores of the highest dose GHBA group (U = 0, P = 0.014), as were those of the 30 mg/kg GHBA group (U = 3, P = 0.014). Both the 60 mg/kg-treated GHBA group scores and the 120 mg/kg-treated GHBA group scores were lower than those of the 240 mg/kg-treated GHBA group. However, the differences only approached significance at P = 0.056.

NIH 10947 (Continued)

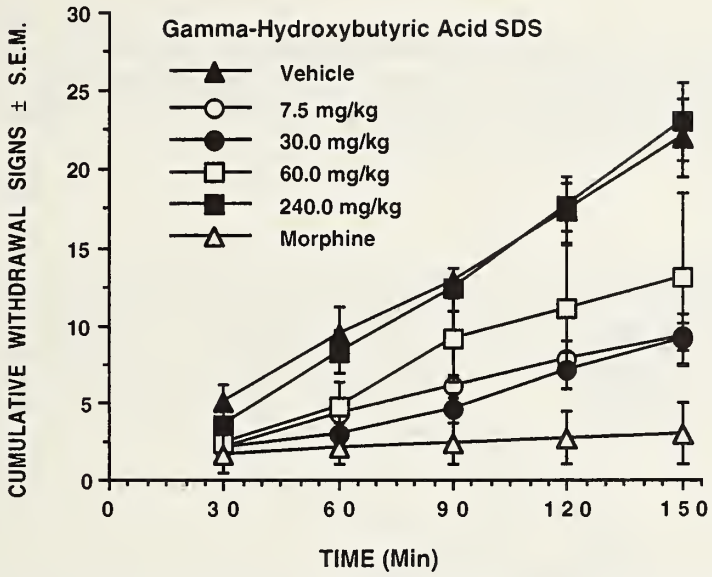
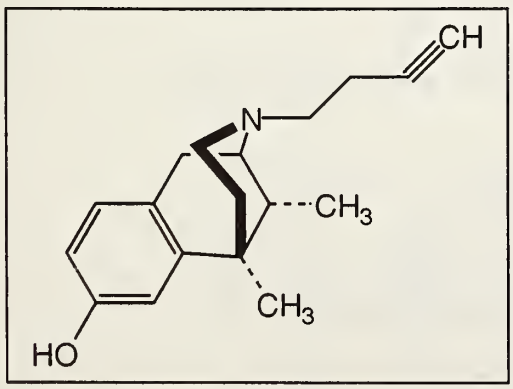


Fig NIH 10947-SDS. Results of study in which single doses of NIH 10947 were substituted for morphine in morphine-dependent monkeys in withdrawal .

Comment: In the mouse and monkey, the results suggest interesting interactions of GHBA with the opioid system which deserve further investigation..

NIH 10963 (±)-2-(3-Butynyl)-5,9α-dimethyl-2'-hydroxy-6,7-benzomorphan
[(±)-N-(3-Butynyl)-N-normetazocine]



MOUSE DATA - ED50 OR AD50

(95 % C.L.) (mg/kg or % change)

1) TF – 0.18 (0.10 – 0.31)
2) TF vs. M – Inactive at 1, 10 and 30
3) PPQ – 0.04 (0.02 – 0.08)
4) HP – 0.14 (0.03 – 0.61)

Special Tests:

1) Naloxone AD50 vs ED80 of NIH 10963 in TF = 0.03 (0.009 – 0.10)
   Opioid subtype tests:
2) β-FNA (i.c.v.) vs ED80 of NIH 10963 (s.c.) in TF: AD50 = 0.53 (0.18 – 1.57) µg/kg.
   Nor-BNI (s.c.) vs ED80 of NIH 10963 (s.c.) in TF: Inactive at 1, 10 and 30 mg/kg.
   c) Naltrindole (s.c.) vs ED80 of NIH 10963 (s.c.) in TF: 0% at 1 and 10 mg/kg, and 24% at 30 mg/kg.

NIH 10963 (Continued)

MONKEY DATA
(SDS)

At the high dose, namely 0.5 mg/kg, this compound substituted completely for morphine (see Fig NIH 10963-SDS). At both doses, 0.125 and 0.5 mg/kg, the morphine agonist signs jaw sag, eyelid ptosis, and slowing were noted. In addition, at the higher dose salivation was seen. The onset was rapid and duration of action was similar to that of morphine. Potency estimate is about six times morphine in this assay.

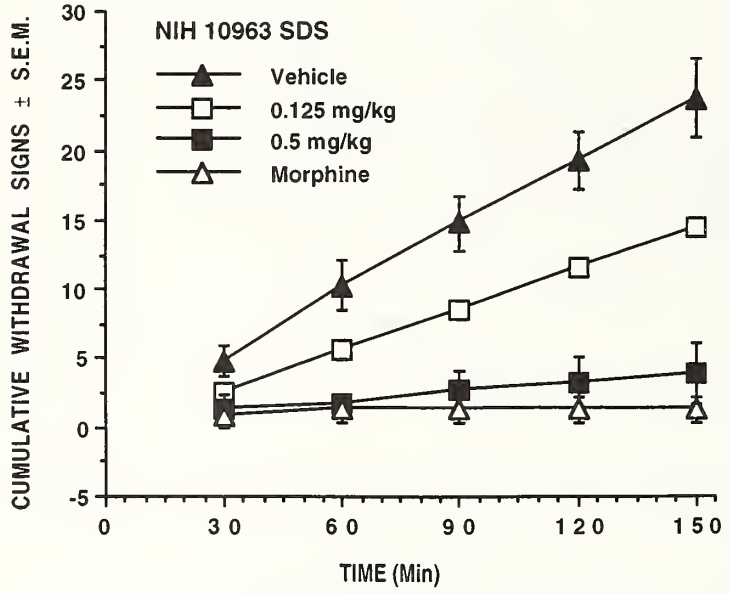
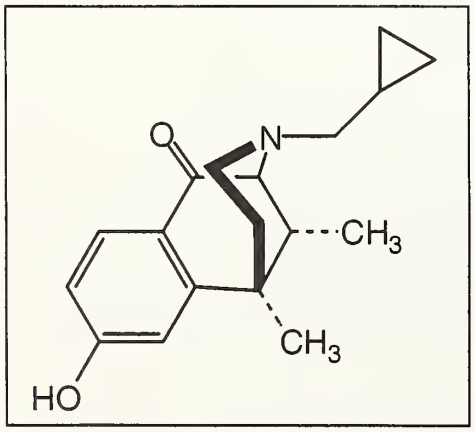


Fig NIH 10963-SDS. Results of study in which single doses of NIH 10963 were substituted for morphine in morphine-dependent monkeys in withdrawal.

Comment: The profile of activity suggested that this compound is a potent mu-opioid receptor agonist.

NIH 10964 (2-Cyclopropylmethyl-5,9α-dimethyl-2'-hydroxy-8-oxo-6,7-benzomorphan)
(±)-8-Ketocyclazocine



MOUSE DATA - ED50 OR AD50
(95 % C.L.) (mg/kg or % change)

1) TF – 0.69 (0.26 – 1.84)
2) TF vs. M – Inactive at 1, 10 and 30
   TF vs. M – Inactive at 1, 10 and 30
3) PPQ – 0.12 (0.05 – 0.28)
4) HP – 0.14 (0.03 – 0.61)

NIH 10964 (Continued)

Special Tests:

1) Opioid subtype tests
   a) Naltrindole (s.c.) vs ED80 NIH 10964 (s.c.) in TF: Inactive at 1, 10 and 30
   b) Nor-BNI (s.c.) vs ED80 of NIH 10964 (s.c.) in TF:Inactive at 1, 10 and 30
   c) β-FNA (i.c.v.) vs ED80 of NIH 10964 (s.c.) in TF: AD50 = 5.66 (1.96 16.33) µg/brain

MONKEY DATA
(SDS)

As shown in the accompanying figure (Fig NIH 10964-SDS), reduced the number of withdrawal signs. However, the reduction was intermixed with the overt signs catalepsy, respiratory depression, salivation and eyelid ptosis, especially at the highest dose. One hr after its administration, one monkey at the highest dose began eating, and appeared normal. In addition, retching was noted in a few monkeys at 0.05 and 0.1 mg/kg. NIH 10964 did not substitute completely for morphine.

MONKEY DATA
(PPT-W)

NIH 10964 dose-dependently precipitated withdrawal in morphine-dependent monkeys. Curiously, during withdrawal, the signs jaw sag, slowing, eyelid ptosis and respiratory depression were noted. Onset and offset were like naloxone's and potency was about 1/4 that of naloxone, the reference standard (see Fig NIH 10964-PPt-Withdrawal).

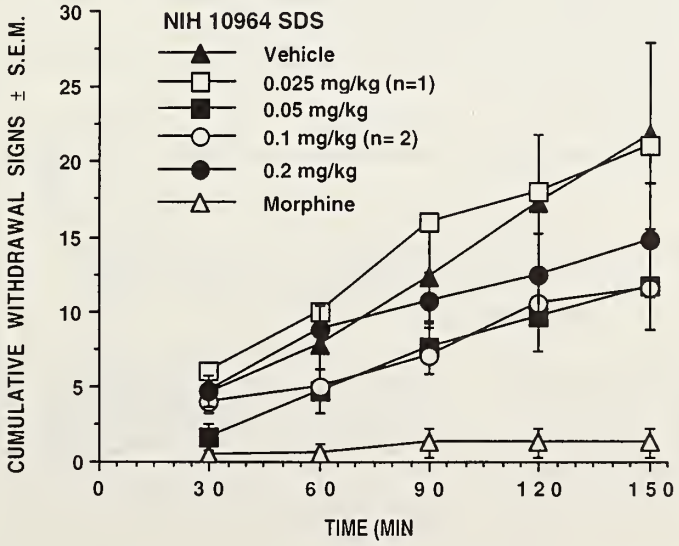


Fig NIH 10964-SDS. Results of study in which single doses of NIH 10964 were substituted for morphine in morphine-dependent monkeys in withdrawal.

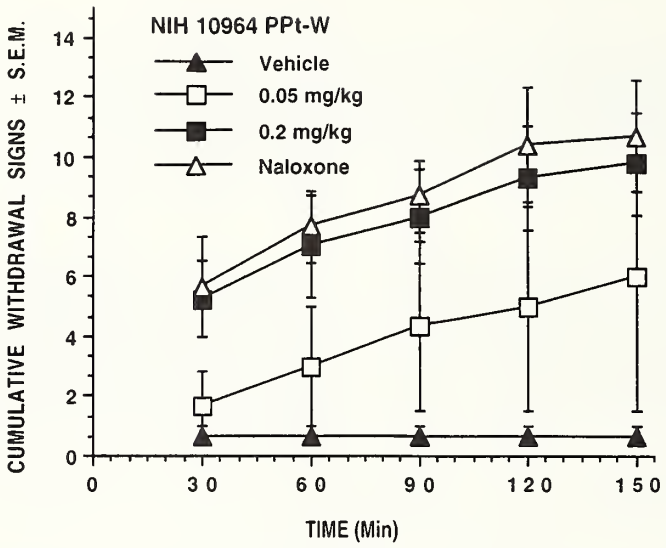
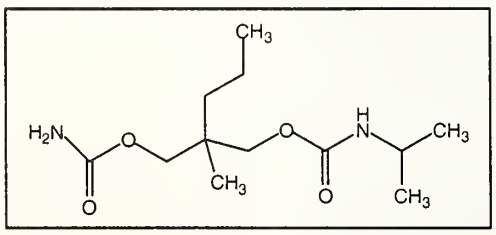


Fig. NIH 10964 -PPt-Withdrawal. Results of study in which morphine-dependent monkeys were given single doses of NIH 10964 two hr after morphine.

Comment: This compound has an unusual profile of activity. In the mouse it appears to be a selective mu-opioid receptor agonist. The effects in morphine-dependent monkeys are reminiscent of those of a partial agonist. When morphine levels are low as in the SDS test, the drug has weak agonist properties. In the presence of morphine it precipitates withdrawal. The behavioral effects suggest kappa-like activity.

NIH 10966 Carisoprodol
((1-Methylethyl)carbamic acid-[[(aminocarbonyl)oxy]methyl]-2-methylpentyl ester)



MOUSE DATA - ED50 OR AD50
(95 % C.L.) (mg/kg or % change)

1) TF – Inactive at 1, 10 and 30[a]
2) TF vs. M – 0% at 1, 5% at 10 and 13% at 30[a]
3) PPQ – 0% at 1, 6% at 10 and 11% at 30[a]
4) HP – 13% at 1, 13% at 10 and 0% at 30[a]

[a]Vehicle was 5% hydroxypropyl-β-cyclodextrin in water.

MONKEY DATA
(SDS)

At doses of 4 and 16 mg/kg, NIH 10966 neither exacerbated nor attenuated withdrawal. No behavioral signs were noted at these doses (see Fig NIH 10966-SDS).

NIH 10966 (Continued)

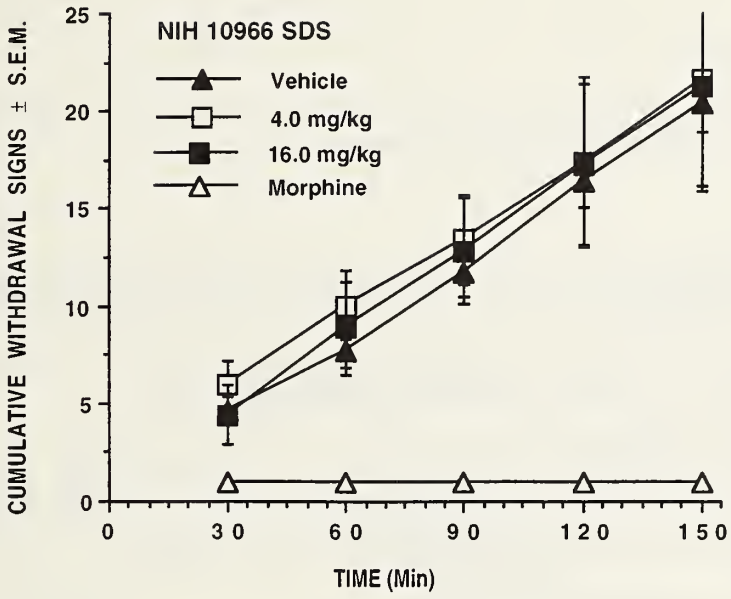
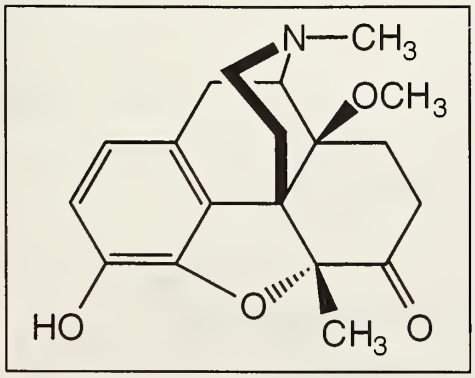


Fig NIH 10966-SDS. Results of study in which single doses of NIH 10966 were substituted for morphine in morphine-dependent monkeys in withdrawal

Comment: At the doses tested, NIH 10966 showed no opioid-like properties.

NIH 10968 (-)-4,5α-Epoxy-3-hydroxy-14β-methoxy-5β,17-dimethylmorphinan-6-one hydrobromide



MOUSE DATA - ED50 OR AD50
(95% C.L.) (mg/kg or % change)

1) TF – 0.03 (0.01 – 0.6)
2) TF vs. M – Inactive at 1, 10 and 30
3) PPQ – 0.009 (0.003 – 0.23)
4) HP – 0.03 (0.01 – 0.05)

Special Tests:

1) Naloxone vs ED80 of NIH 10968 in TF: AD50 = 0.02 (0.01 – 0.05)
2) Opioid subtype tests
   a) β-FNA (i.c.v.) vs ED80 of NIH 10968 (s.c.) in TF: AD50 = 0.55 μg/brain.
   b) Nor-BNI (s.c.) vs ED80 of NIH 10968 (s.c.) in TF: Inactive at 1, 10 and 30.
   c) Naltrindole (s.c.) vs ED80 of NIH 10968 (s.c.) in TF: Inactive at 1, 10 and 30

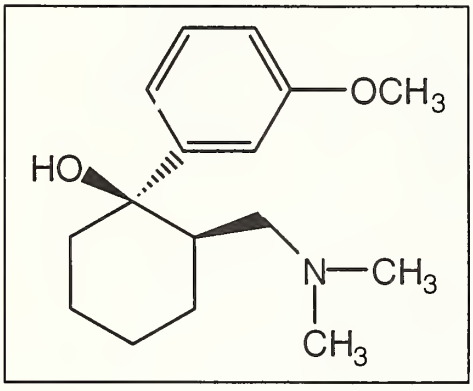
NIH 10968 (Continued)

MONKEY DATA
(SDS)

Not Tested.

Comment: Apparently, NIH 10968 is a mu-opioid receptor agonist in the mouse.

NIH 10969 Tramadol hydrochloride
trans-(±)-2-[(Dimethylamino)methyl]-1-(3-methoxyphenyl)cyclohexanol hydrochloride



MOUSE DATA - ED50 OR AD50
(95 % C.L.) (mg/kg or % change)

1) TF – 1% at 1, 7% at 10 and 18% at 30
2) TF vs. M – 4% at 1, 30% at 10 and 24% at 30
3) PPQ – 6.13 (2.41 – 15.58)
4) HP – 0% at 1, 13% at 10 and 30

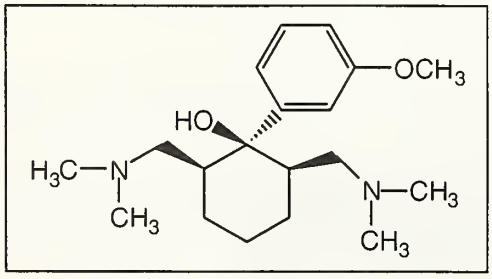
Special Test:
1) Opioid subtype test
   a) Naltrindole (s.c.) vs ED80 of NIH 10969 (s.c.) in PPQ:
   Inactive at 1, 10 and 30 mg/kg

MONKEY DATA
(SDS)

Not Tested.

Comment: Antinociceptive activity in the mouse was not delta-opioid receptor-related.

NIH 10970 2,6-Bis[(dimethylamino)methyl]-1-(3-methoxyphenyl)cyclohexanol dihydrochloride



MOUSE DATA - ED50 OR AD50
(95 % C.L.) (mg/kg or % change)

1) TF –Inactive at 1, 10 and 30
2) TF vs. M – Inactive at 1, 10 and 30
3) PPQ – 3% at 1, 9% at 10 and 14% at 30
4) HP – Inactive at 1, 10 and 30

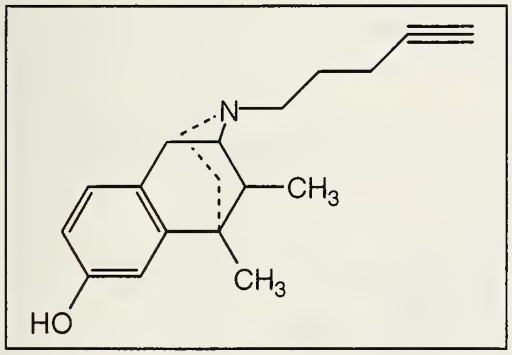
NIH 10970 (Continued)

MONKEY DATA
(SDS)

Not Tested.

Comment: Based on the results in mice, NIH 10970 appears devoid of opioid agonist and antagonist antinociceptive activity.

NIH 10971 (+)-(1*S*,5*S*,9*S*)-5,9-dimethyl-2'-hydroxy-2-(4-pentynyl)-6,7-benzomorphan dihydrochloride



MOUSE DATA - ED50 OR AD50
(95 % C.L.) (mg/kg or % change)

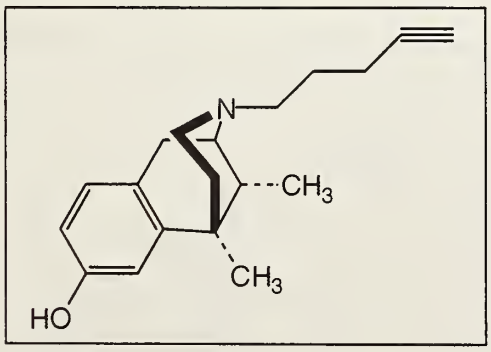
1) TF – Inactive at 1, 10 and 30[a,b]
2) TF vs. M – Inactive at 1, 10 and 30[b]
3) PPQ – 14.77 (11.17 - 19.53)[b]
4) HP – Inactive at 1, 10 and 30[a]
[a]Ataxia at 30.
[b]Vehicle - Dilute HCl.

MONKEY DATA
(SDS)

Not Tested.

Comment: The results suggest that NIH 10971 has weak antinociceptive activity in the PPQ test.

NIH 10972 (-)-(1*R*,5*R*,9*R*)-5,9-dimethyl-2'-hydroxy-2-(4-pentynyl)-6,7-benzomorphan



MOUSE DATA - ED50 OR AD50
(95 % C.L.) (mg/kg or % change)

1) TF – Inactive at 1, 10 and 30[a,b]
2) TF vs. M – 2.03 (1.13 - 3.65)[b]
3) PPQ – 11.78 (4.65 - 29.84)[b]
4) HP – 0% at 1, 13% at 10 and 0% at 30[a,b]
[a]Ataxia at 30.
[b]Vehicle - Dilute HCl

NIH 10972 (Continued)

Special Tests:

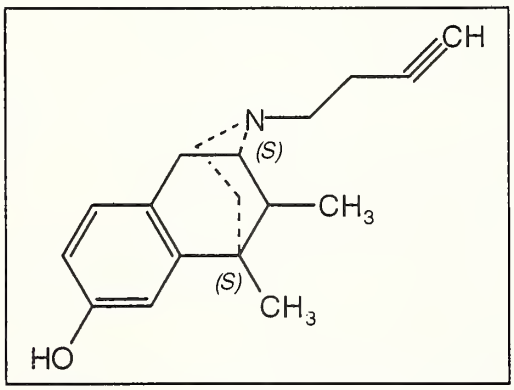
1) Opioid subtype tests
   a) Naltrindole vs ED80 of NIH 10972 in PPQ: Inactive at 1, 10 and 30.
   b) β-FNA (i.c.v., 240 min pretreatment time) vs ED80 of NIH 10972 (s.c., 20 min pretreatment time) in PPQ test: 14% at 1, 20% at 3, 43% at 10 and 17% at 30 µg/brain.
   c) Nor-BNI (s..c., 120 pretreatment time) vs ED80 of NIH 10972 (s.c.), 20 min pretreatment time) in PPQ test: Inactive at 1, 3, 10 and 30.
   d) ED80 of U-69,593 (i.c.v.) 20 min pretreatment time) vs NIH 10972 (s.c., 30 min pretreatment time) in TF test: AD50 = 1.24 (0.48 - 3.25)
   e) ED80 of DPDPE (i.c.v., 10 min pretreatment time) vs NIH 10972 (s.c, 30 min pretreatment time) in TF test: AD50 = 0.05 (0.02 - 0.11).

MONKEY DATA
(SDS)

Not Tested.

Comment: NIH 10972 has agonist/antagonist antinociceptive properties. It appears to have weak mu-opioid receptor agonist effects as well as mu-, kappa- and delta-opioid receptor antagonist properties.

NIH 10973 (+)-(1*S*,5*S*,9*S*)-2-(3-Butynyl)-5,9-dimethyl-2'-hydroxy-6,7-benzomorphan .HCl



MOUSE DATA - ED50 OR AD50
(95 % C.L.) (mg/kg or % change)

1) TF – Inactive at 1, 10 and 30[a]
2) TF vs. M – Inactive at 1, 10 and 30
3) PPQ – 6% at 1, 14% at 10 and 23% at 30
4) HP – Inactive at 1, 10 and 30[a]

[a]Increased locomotor activity at 10 and 30 and ataxia at 30.

MONKEY DATA
(SDS)

NIH 10973 (Continued)

As shown in the accompanying figure, NIH 10973-SDS, produced a non dose-related attenuation of withdrawal signs. At the highest dose, the reduction in scores was accompanied by the signs designated as ataxia, slowing, jaw sag and salivation. In a preliminary study, the monkey's behavior was described as disoriented and ketamine-like. Muscle fasciculations were also noted.

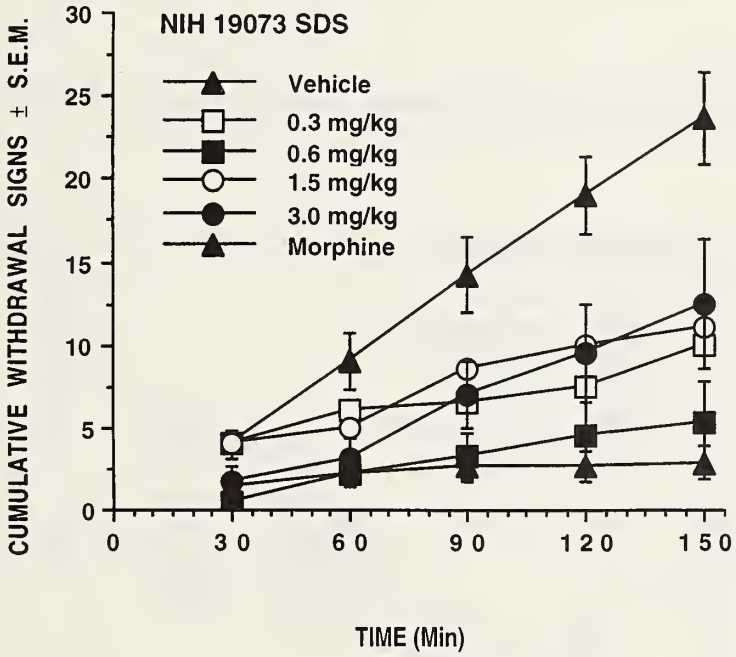
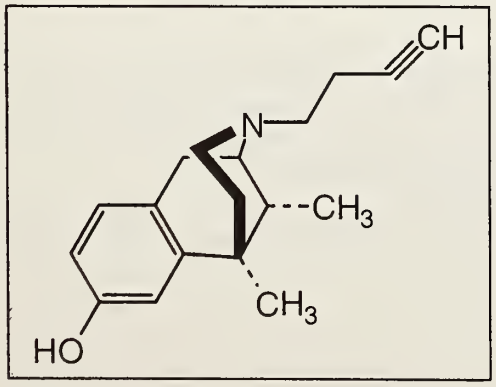


Fig NIH 10973-SDS. Results of study in which single doses of NIH 10973 were substituted for morphine in morphine-dependent monkeys in withdrawal.

Comment: The results in mice and monkeys suggest non opioid-receptor related CNS properties.

NIH 10974 (-)-(1*R*,5*R*,9*R*)-2-(3-Butynyl)- 5,9-dimethyl-2'-hydroxy-6,7-benzomorphan .HCl



MOUSE DATA - ED50 OR AD50
(95 % C.L.) (mg/kg or % change)

1) TF – 0.05 (0.03 - 0.09)
2) TF vs. M – Inactive at 1, 10 and 30[a]
3) PPQ – 0.04 (0.02 - 0.08)
4) HP – 0.13 (0.05 - 0.37)

[a]Loss of righting reflex at 10 and 30.

NIH 10974 (Continued)

Special Tests:

1) Naloxone vs ED80 of NIH 10974 in TF: AD50 = 0.03 (0.009 - 0.09).
2) Opioid subtype tests
   a) Nor-BNI (s.c.) vs ED80 of NIH 10974 (s.c.) in TF: AD50 = 0.29 (0.09 - 0.93).
   b) Naltrindole (s.c.) vs ED80 of NIH 10974 (s.c.) in TF: AD50 = 3.11 (1.02 - 9.42).
   c) β-FNA (i.c.v.) vs ED80 of NIH 10974 (s.c.) in TF: AD50 = 0.64 (0.32 - 1.31).

MONKEY DATA

(SDS)

This compound produced a dose-related attenuation of withdrawal signs (see Fig NIH 10974-SDS). At the highest dose, jaw sag, ataxia, body sag and slowing were also noted.

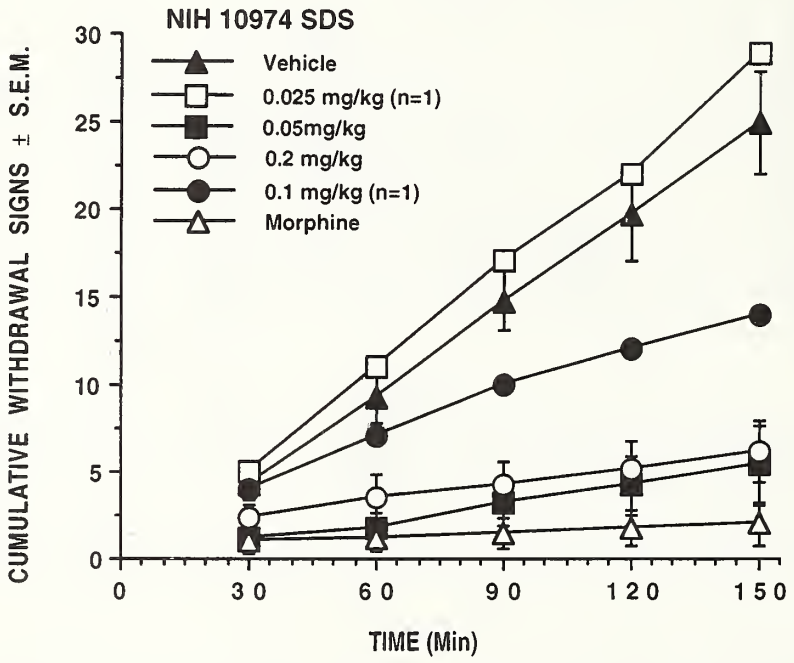
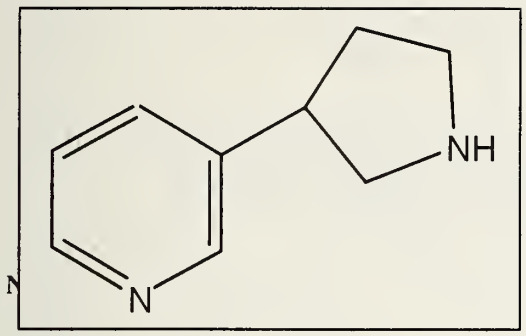


Fig NIH 10974-SDS. Results of study in which single doses of NIH 10974 were substituted for morphine in morphine-dependent monkeys in withdrawal.

Comment: The evidence suggests that NIH 10974 appears to be a non-selective mu, kappa and delta opioid agonist in the mouse and monkey. Curiously, this is not reflected in the rather low naloxone AD50 versus the ED80 of this compound in the tail-flick test.

NIH 10975 (-)-N-Norisonicotine di-l-tartrate



MOUSE DATA - ED50 OR AD50
(95 % C.L.) (mg/kg or % change)

1) TF – Inactive at 1, 10 and 30
2) TF vs. M – Inactive at 1, 10 and 30
3) PPQ – Inactive at 1 and 10, 17% at 30
4) HP – Inactive at 1, 10 and 30

MONKEY DATA
(SDS)

Some weak non dose-related attenuation of withdrawal scores in rhesus monkeys was noted (see Fig NIH 10975-SDS)).

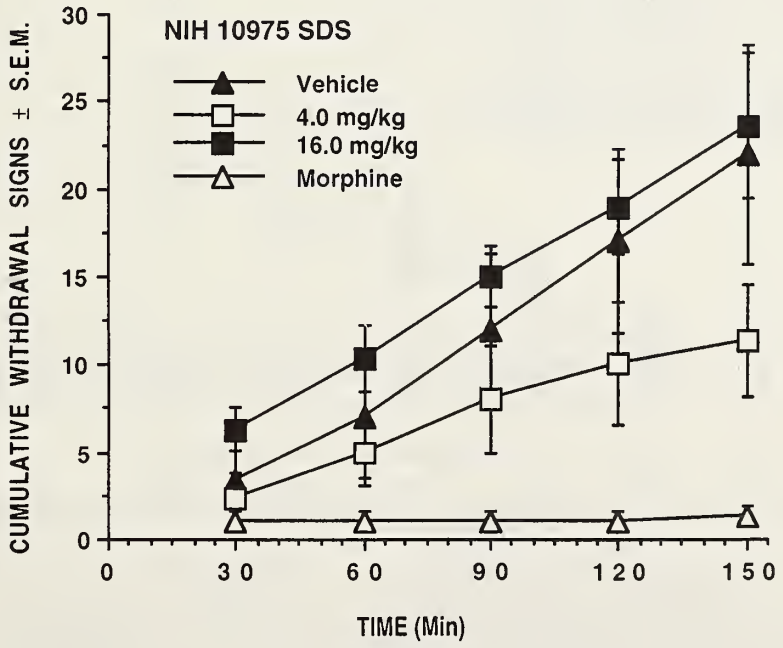
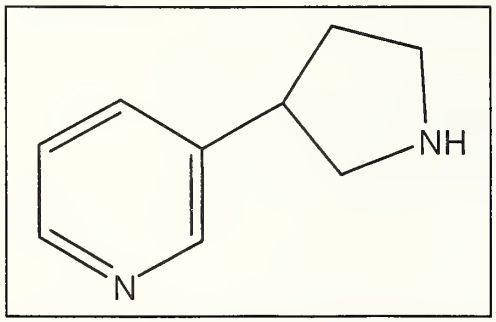


Fig NIH 10975-SDS. Results of study in which single doses of NIH 10975 were substituted for morphine in morphine-dependent monkeys in withdrawal.

Comment: The profile of activity does not predict opioid receptor interactions.

NIH 10976 (+)-N-Norisonicotine di-d-tartrate



MOUSE DATA - ED50 OR AD50
(95 % C.L.) (mg/kg or % change)

1) TF – Inactive at 1, 10 and 30
2) TF vs. M – 13% at 1, 0% at 10 and 10% at 30
3) PPQ – 11% at 1, 9% at 10 and 29% at 30
4) HP – Inactive at 1, 10 and 30

MONKEY DATA
(SDS)

As shown in the Fig NIH 10976-SDS below, at the high dose it appears that NIH 10976 exacerbated withdrawal. However, the increased withdrawal score is not significantly different from control.

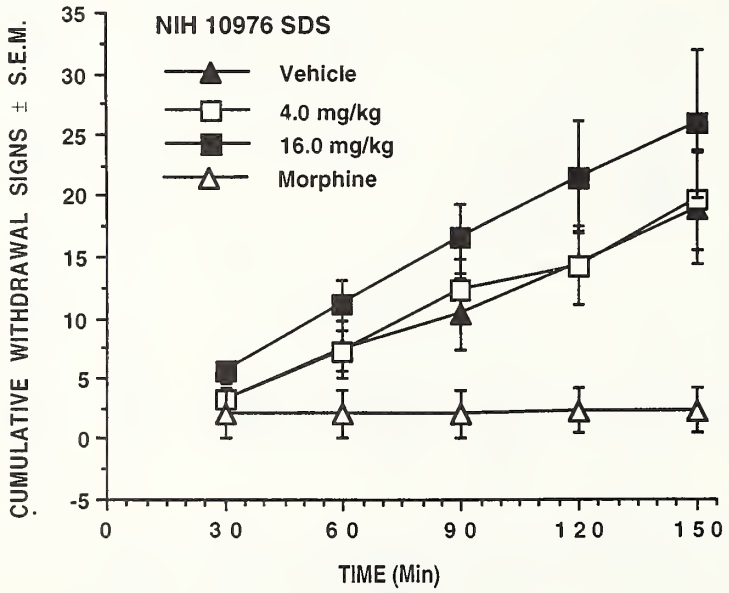
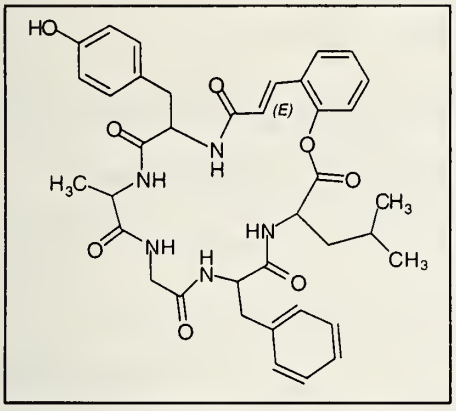


Fig NIH 10976-SDS. Results of study in which single doses of NIH 10976 were substituted for morphine in morphine-dependent monkeys in withdrawal.

Comment: Neither the mouse nor the monkey data are indicative of opioid activity.

NIH 10977 Coumarin-based cyclic prodrug of DADLE



A. Original Sample

MOUSE DATA - ED50 OR AD50
(95 % C.L.) (mg/kg or % change)

1) TF – Inactive at 1 and 10, 1% at 30[a]
2) TF vs. M – 3% at 1, 8% at 10 and 18% at 30[a,b]
3) PPQ – 32.50 (19.69 - 53.66)[a]
4) HP – Inactive at 1, 25% at 10 and 30[a]

[a]Vehicle was 35% hydroxypropyl-β-cyclodextrin in water.
[b]Vehicle - 8% antagonism

Special Test: Naltrindole vs ED80 of NIH 10977 in PPQ test: Inactive at 1 and 10, 22% at 30.

B. New Sample (Stored at -70° C)

TF - (i.v., 90 min pretreatment) Inactive at 1, 10 and 30[a,b]
TF - (s.c., 90 min pretreatment) Inactive at 1, 10 and 30[a]
TF - (s.c., 90 min pretreatment) 6% at 1, 17% at 10 and 9% at 30[a]

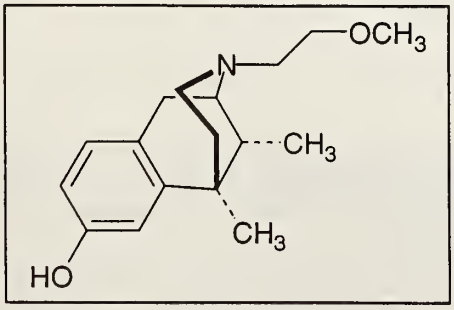
[a]Vehicle was 35% hydroxypropyl-β-cyclodextrin.
[b] Eyelid ptosis in 2/6 mice, mice immobile at 30 mg/kg

MONKEY DATA
(SDS)

Not Tested.

Comment: The antinociceptive profile with the original sample indicated weak activity in the PPQ and HP tests. The special naltrindole test detected weak delta-opioid receptor-related antinociception. The new sample appeared inactive, antinociceptively, when given intravenously 90 min before testing.

NIH 10980 (-)-(1*R*,5*R*,9*R*)-5,9-Dimethyl-2'-hydroxy-2-(2-methoxyethyl)-6,7-benzomorphan.HCl



MOUSE DATA - ED50 OR AD50
(95 % C.L.) (mg/kg or % change)

1) TF – 0.072 (0.03 - 0.17)[a]
2) TF vs. M – 0% at 1 and 10, 19% at 30[b]
3) PPQ – 0.01 (0.004 - 0.021)
4) HP – 0.15 (0.05 - 0.44)

[a] Moderate Straub tails at 0.5 mg/kg.
[b] Ataxia at 10 mg/kg.

NIH 10980 (Continued)

Special Tests:

1) Naloxone vs ED80 of NIH 10980 in TF: AD50 = 0.28 (0.11 - 0.74).
2) Opioid subtype tests
   a. Nor-BNI (s.c., 120 min pretreatment time) vs ED80 of NIH 10980 (s.c., 20 min pretreatment time) in TF test: Inactive at 1, 10 and 30.
   b. Naltrindole (s.c., 20 min pretreatment time) vs ED80 of NIH 10980 (s.c., 20 min pretreatment time) in TF test: 1% at 1 and 10, 13% at 30.
   c. β-FNA (i.c.v., 240 min pretreatment time) vs ED80 of NIH 10980 (s.c., 20 min pretreatment time) in TF: AD50 = 3.13 (1.21 - 8.12) mg/kg.

MONKEY DATA
(SDS)

In morphine-dependent monkeys in withdrawal, at doses of 0.00325, 0.0125, 0.05 and 0.2 mg/kg NIH 10980 produced a non dose-related attenuation of withdrawal signs ((see Fig NIH 10980-SDS). At 0.05 mg/kg, the sign jaw sag was observed. The signs jaw sag, ataxia, slowing, salivation and eyelid ptosis were seen at 0.2 mg/kg. Most of the attenuation of withdrawal signs was attributable to abdominal muscle relaxation.

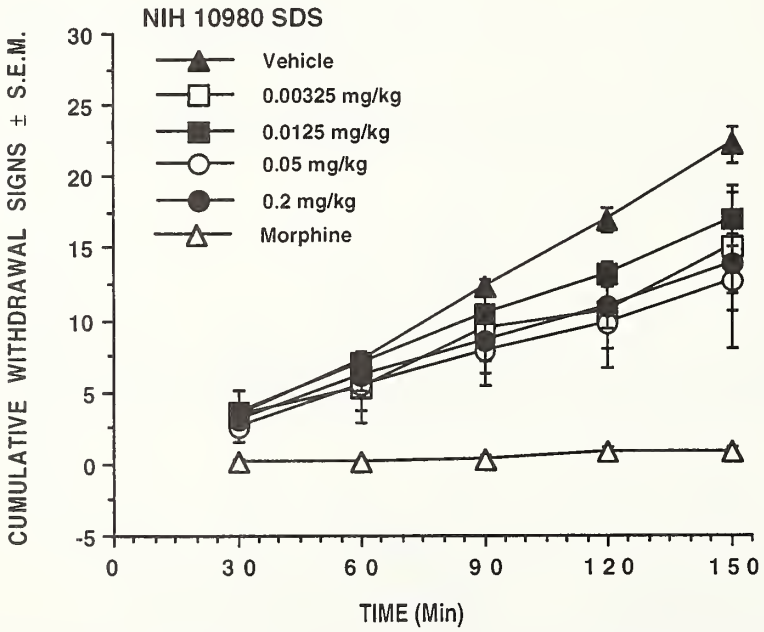
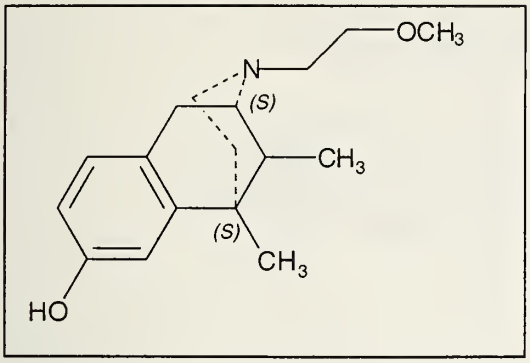


Fig NIH 10980-SDS. Results of study in which single doses of NIH 10980 were substituted for morphine in morphine-dependent monkeys in withdrawal.

Comment: Although the data in the mouse indicate mu-opioid antinociceptive properties, the results in monkeys were not consistent with this finding, i.e., at no dose did NIH 10980 substitute completely for morphine even at doses in which the overt behavioral signs suggested mu- or kappa-opioid effects.

NIH 10981 (+)-(1*S*,5*S*,9*S*)-5,9-Dimethyl-2'-hydroxy-2-(2-methoxyethyl)-6,7-benzomorphan.HCl



MOUSE DATA - ED50 OR AD50
(95 % C.L.) (mg/kg or % change)

1) TF – Inactive at 1, 10 and 30
2) TF vs. M – 10% at 1 and 10, 11% at 30
3) PPQ – 22.71 (10.94 - 47.12)[a]
4) HP – 0% at 1, 13% at 10 and 30

[a]Ataxia, increased locomotor activity and moderate Straub tails at 60 mg/kg.

MONKEY DATA
(SDS)

Insufficient supplies of NIH 10981 precluded a full study of this compound. Nevertheless, limited testing (n=1/dose) indicated that at the high dose of 12 mg/kg, it partially suppressed withdrawal (see Fig NIH 10981-SDS). However, this effect was accompanied by the signs ataxia and slowing. Onset of action for these behavioral signs was prompt (within 30 min).

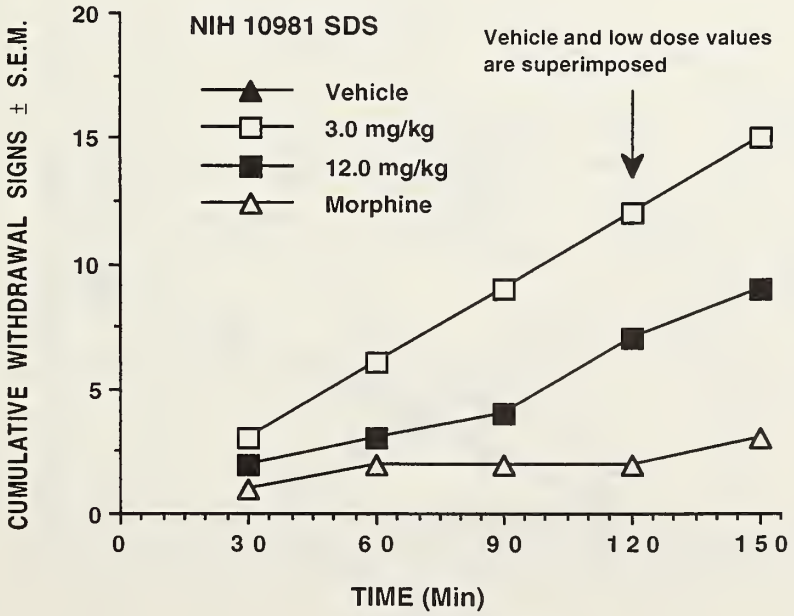
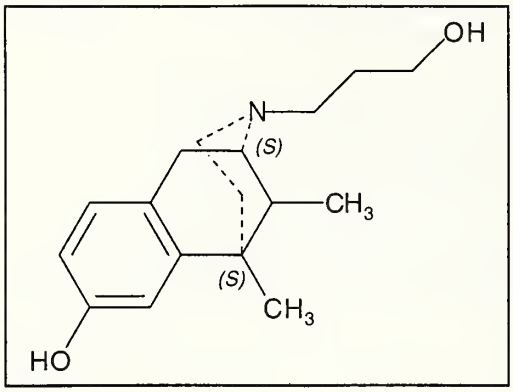


Fig NIH 10981-SDS. Results of study in which single doses of NIH 10981 were substituted for morphine in morphine-dependent monkeys in withdrawal.

Comment: NIH 10981 showed weak antinociceptive activity in the PPQ test. Effects in the morphine-dependent monkey while inconclusive indicate some suppression of withdrawal at the high dose accompanied by the signs ataxia and slowing. Perhaps this compound has mixed opioid agonist and other CNS properties.

10982 (+)-(1*S*,5*S*,9*S*)-5,9-Dimethyl-2'-hydroxy-2-(3-hydroxypropyl)-6,7-benzomorphan.HCl



MOUSE DATA - ED50 OR AD50
(95 % C.L.) (mg/kg or % change)

1) TF – Inactive at 1 and 10, 7% at 30
2) TF vs. M – 9% at 1, 0% at 10 and 2% at 30
3) PPQ – 0% at 1, 3% at 10 and 20% at 30
4) HP – 0% at 1 and 10, 13% at 30

MONKEY DATA
(SDS)

As shown in the figure, at 4 and 16 mg/kg, NIH 10982 partially substituted for morphine. The effect was not dose-related and was more apparent during the first 60 min suggesting that duration of action is short (see Fig NIH 10982-SDS). This could be due to metabolism and/or rapid elimination of the compound.

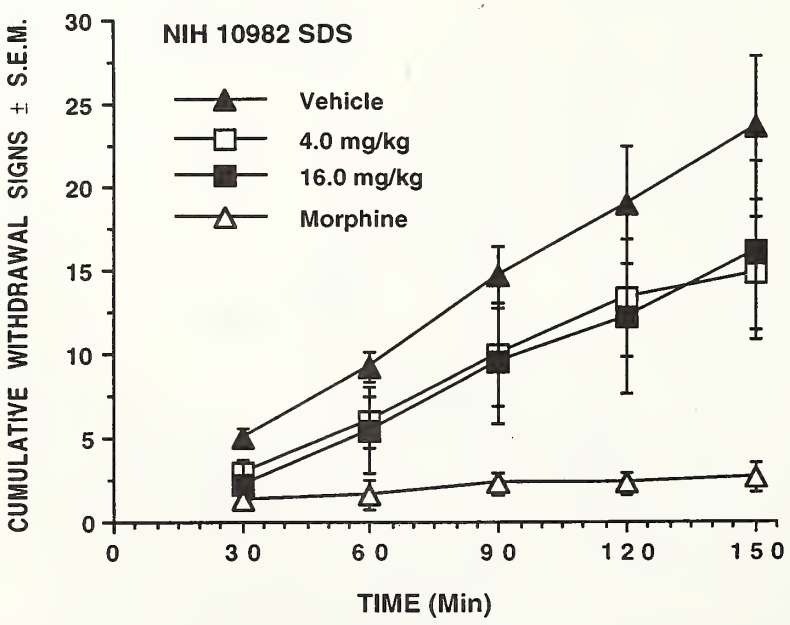
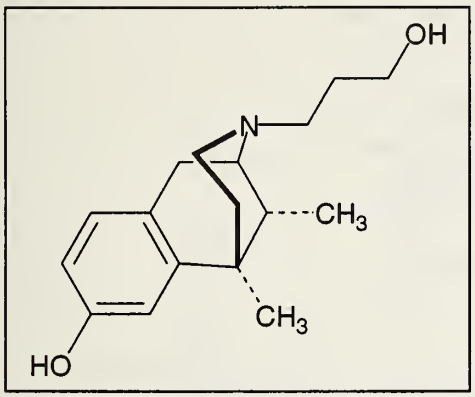


Fig NIH 10982-SDS. Results of study in which single doses of NIH 10982 were substituted for morphine in morphine-dependent monkeys in withdrawal .

Comment: The results in mice and monkeys do not predict potent opioid effects.

NIH 10983 (-)-(1*R*,5*R*,9*R*)-5,9-Dimethyl-2'-hydroxy-2-(3-hydroxypropyl)-6,7-benzomorphan.HCl



MOUSE DATA - ED50 OR AD50
(95 % C.L.) (mg/kg or % change)

1) TF – Inactive at 1, 10 and 30
2) TF vs. M – 0% at 1, 11% at 10, and 33% at 30
3) PPQ – 11.64 (3.48 - 38.98)
4) HP – Inactive at 1, 10 and 30

MONKEY DATA
(SDS)

At 16 mg/kg NIH 10983 exacerbated withdrawal (see Fig NIH 10983-SDS). Onset was prompt and duration of action was at least 2.5 hr. At this dose some ataxia was observed.

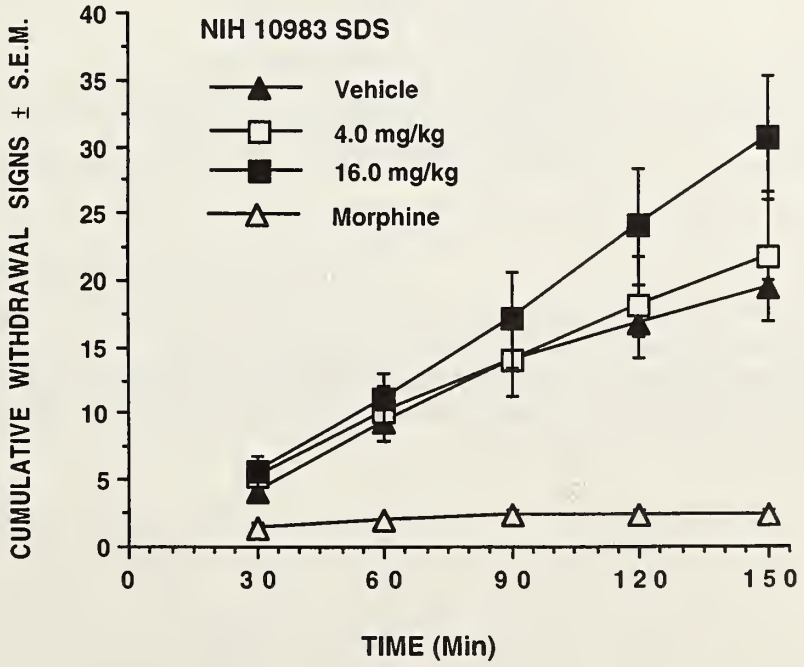
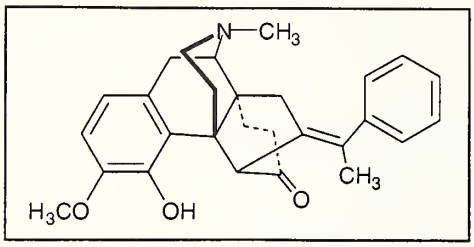


Fig NIH 10983-SDS. Results of study in which single doses of NIH 10983 were substituted for morphine in morphine-dependent monkeys in withdrawal .

Comment: The results in the mouse and monkey are supportive of weak mu-opioid antagonist activity. Some agonist antinociceptive activity is evident in the mouse PPQ test.

NIH 10984 E-3-Methoxy-4-hydroxy-5,14-ethano-18-(1-methyl)benzylidene-6-oxo-N-methylmorphinan



MOUSE DATA - ED50 OR AD50
(95 % C.L.) (mg/kg or % change)

1) TF – 0.05 (0.02 - 0.09)[a]
2) TF vs. M – Inactive at 1 and 10[a]
3) PPQ – 0.012 (0.005 - 0.031)[a]
4) HP – 0.1 (0.04 - 3.24)[a]

[a]Vehicle was 10% DMSO in water.

Special Tests: 1) Opioid subtype tests

a. β-FNA (i.c.v.) vs ED80 of NIH 10984 (s.c.) in TF: AD50 = 1.23 (0.47 - 3.24) μ/brain.
b. nor-BNI (s.c.) vs ED80 of NIH 10984 (s.c.) in TF: Inactive at 1, 10 and 30.
c. Naltrindole (s.c.) vs ED80 of NIH 10984 (s.c.) in TF: Inactive at 1 and 10, 28% at 30.

Table 1. Study of the antagonist effects of NIH 10984 versus the ED80 morphine, a mu-opioid receptor agonist, using the tail-flick test.

NIH 10984: Pretreatment time (hr)	**AD50 or % antagonism versus ED80 of Morphine·SO_4**
2	Inactive at 1, 3, 10 and 30 mg/kg
6	Inactive at 1, 3, 10 and 30 mg/kg
24	Inactive at 1, 3, 10 and 30 mg/kg
48	Inactive at 1, 3, 10 and 30 mg/kg
72	22% at 1; 18 % at 3; 0 % at 10 and 15% at 30
96	Inactive at 1, 3, 10 and 30
120	Inactive at 1, 3, 10 and 30

MONKEY DATA
(SDS)

As illustrated in Fig NIH 10984-SDS, this compound substituted completely for morphine. The effect was dose-related and onset and duration of action were similar to morphine. The potency estimate was at least 100 times that of morphine. At the high dose, signs such as jaw sag, ataxia, eyelid ptosis and scratching were observed. This syndrome is commonly seen with doses of morphine that are higher than that necessary to substitute for morphine. Vehicle was 10% hydroxypropyl-β-cyclodextrin in water.

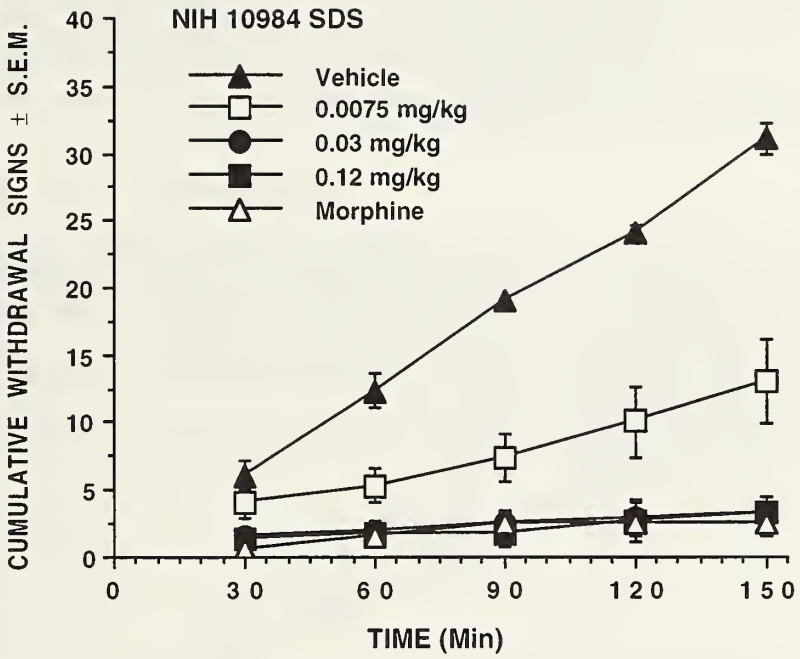


Fig NIH 10984-SDS. Results of study in which single doses of NIH 10984 were substituted for morphine in morphine-dependent monkeys in withdrawal

Comment: The results in the mouse and morphine-dependent monkey show a profile of activity that is consistent with that of a potent mu-opioid receptor agonist. Pretreatment in the mouse with NIH 10984 for up to 120 hr does not reveal opioid antagonist properties with this compound.

NIH 10991 (*R*)-5-(2-Azetidinylmethoxy)-2-chloropyridine .*p*-toluenesulfonate

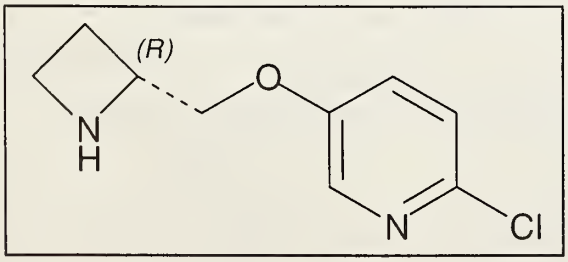


TABLE A. Effects of NIH 10991 in the tail-flick- and paraphenylquinone-antinociceptive tests.

Test	Route of Administration	Data Expressed as ED80 or AD50 (95% CL.) (mg/kg or % change)
TF	s.c. (20 min pretreatment)	8% at 1, 34% at 10[a,b]
PPQ	s.c. (10 min pretreatment before morphine)	0.004 (0.002 - 0.008)[a,b]

[a]Two of 6 convulsed and died at 10; [b]Tremors and immobility at 10.

Special Tests:
Opioid subtype tests

a) β-FNA (mu antagonist, i.c.v., 2 hr pretreatment) vs ED80 of NIH 10991 (s.c., 20 min pretreatment) in PPQ test: 3% at 1 µg/brain, 0% at 3 µg/brain, 24% at 10 µg/brain and 57% at 30 µg/brain.

b) nor-BNI (kappa antagonist, s.c., 2 hr pretreatment) vs ED80 of NIH 10991 (s.c., 20 min pretreatment) in PPQ test: AD50 = 11.34 (5.25 - 24.49) mg/kg.

c) Naltrindole (s.c., 30 min pretreatment) vs ED80 of NIH 10991 (s.c., 20 min pretreatment) in PPQ test: 5% at 1 and 10, 48% at 30 and 54% at 60.

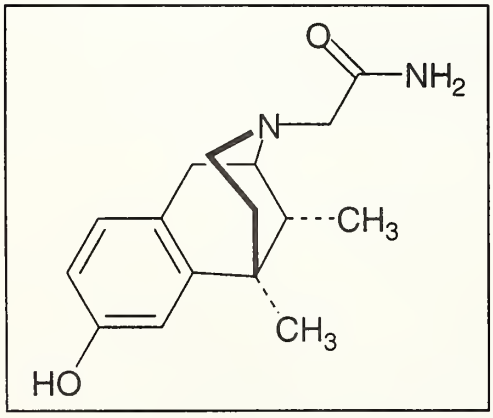
d) Mecamylamine (s.c., 30 min pretreatment) vs ED80 of NIH 10991 (s.c., 20 min pretreatment) in PPQ test: 7% at 1 and 10.

MONKEY DATA
(SDS)

Not Tested.

Comment: This compound was very potent in the PPQ test and weakly active in the tail flick test. Weak but definite dose-related mu, kappa and delta-opioid properties were evident. Lack of activity with mecamylamine indicates the antinociception in the PPQ test is not nicotine related.

NIH 10993 (-)-(1*R*,5*R*,9*R*)-2-Acetamido-5,9-dimethyl-2'-hydroxy-6,7-benzomorphan



MOUSE DATA - ED50 OR AD50
(95 % C.L.) (mg/kg or % change)

1) TF – Inactive at 1, 10 and 30[a]
2) TF vs. M – 11% at 1, 0% at 10 and 15% at 30[a]
3) PPQ – 3.45 (1.42 - 8.41)[a.]
4) HP – 13% at 1 and 10, 0% at 30[a]

[a]Vehicle was 5% hydroxypropyl-β-cyclodextrin in water.

NIH 10993 (Continued)

MONKEY DATA
(SDS)

The data illustrated in Fig NIH 10993-SDS indicate that this compound neither substituted for morphine at 4 and 16 mg/kg nor exacerbated withdrawal. Dilute HCl was used to dissolve the compound.

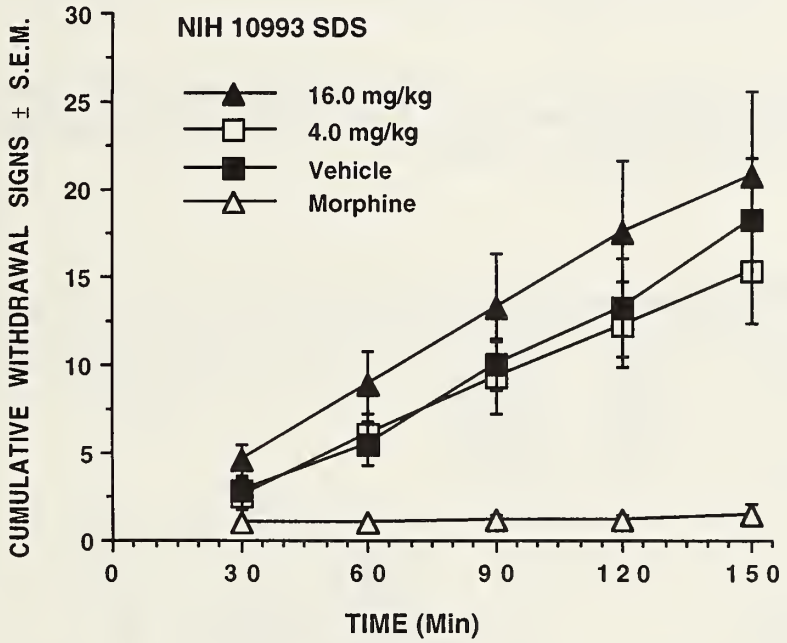
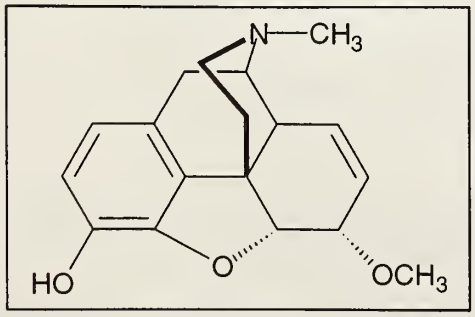


Fig NIH 10993-SDS. Results of study in which single doses of NIH 10993 were substituted for morphine in morphine-dependent monkeys in withdrawal.

Comment: Except for some weak antinociceptive activity in the PPQ test, NIH 10993 does not display a profile of activity indicative of mu-opioid agonists or antagonists.

NIH 10998 Heterocodeine hydrochloride



MOUSE DATA - ED50 OR AD50
(95 % C.L.) (mg/kg or % change)

1) TF – 0.21 (0.12 - 0.38)
2) TF vs. M – Inactive at 1, 10 and 30
3) PPQ – 0.04 (0.02 - 0.10)
4) HP – 0.51 (0.30 - 0.87)

NIH 10998 (Continued)

Special Tests:

1) Naloxone vs ED80 of NIH 10998 in TF: AD50 = 0.04 (0.02 - 0.10).
2) Opioid subtype tests:
   a) β-FNA (i.c.v.) vs ED80 of NIH 10998 (s.c.) in TF: AD50 = 0.06 μg/brain.
   b) Nor-BNI (s.c.) vs ED80 of NIH 10998 (s.c.) in TF: Inactive at 1 and 10, 3% at 30.
   c) Naltrindole (s.c.) vs ED80 of NIH 10998 (s.c.) in TF: 11% at 1, 29% at 10 and 39% at 30.

MONKEY DATA

(SDS)

As depicted in the Fig NIH 10998-SDS, this compound substituted for morphine in a dose-related manner. Onset of action was prompt and effect lasted for at least 2.5 hr. At the high dose, body and jaw sag, and ataxia were observed. The potency estimate is 2 times that of morphine.

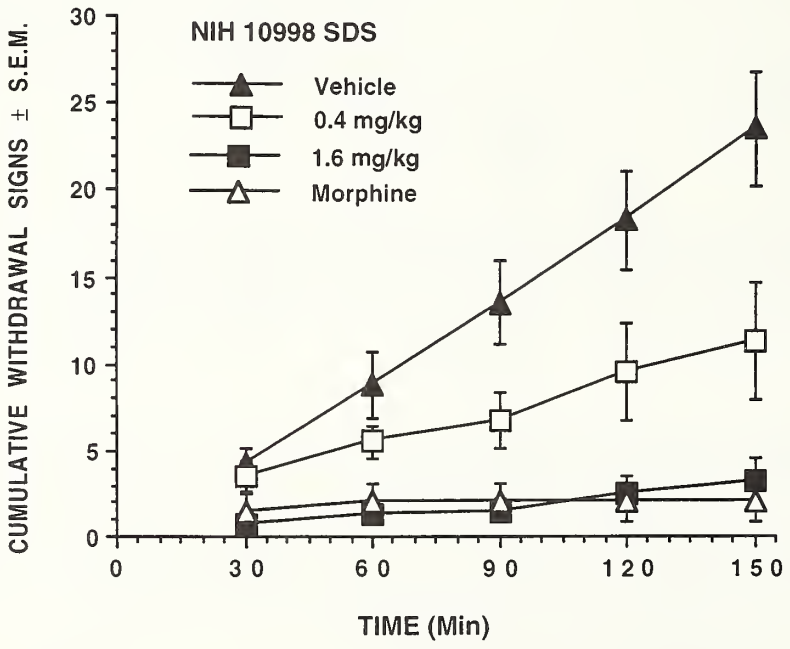
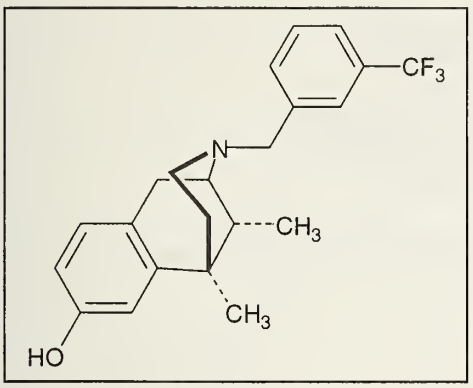


Fig NIH 10998-SDS. Results of study in which single doses of NIH 10998 were substituted for morphine in morphine-dependent monkeys in withdrawal.

Comment: NIH 10998 is a potent mu-opioid receptor agonist with some weak delta receptor activity.

NIH 10999 (-)-(1*R*,5*R*,9*R*)-5,9-Dimethyl-2'-hydroxy-2-(3-trifluoromethylbenzyl)-6,7-benzomorphan hydrochloride



MOUSE DATA - ED50 OR AD50
(95 % C.L.) (mg/kg or % change)
1) TF – 0% at 1, 5% at 10 and 0% at 30[a]
2) TF vs. M – Inactive at 1, 10 and 30[a]
3) PPQ – 6% at 1, 9% at 10 and 26% at 30[a]
4) HP – Inactive at 1, 10 and 30[a]

[a]Vehicle was 5% hydroxypropyl-β-cyclodextrin in water.

MONKEY DATA
(SDS)

The results displayed in Fig NIH 10999-SDS suggest that this compound substituted briefly for morphine. However, activity was waning by 90 min. However, drug supply was exhausted precluding a full study. Vehicle was 10% hydroxypropyl-β-cyclodextrin in water.

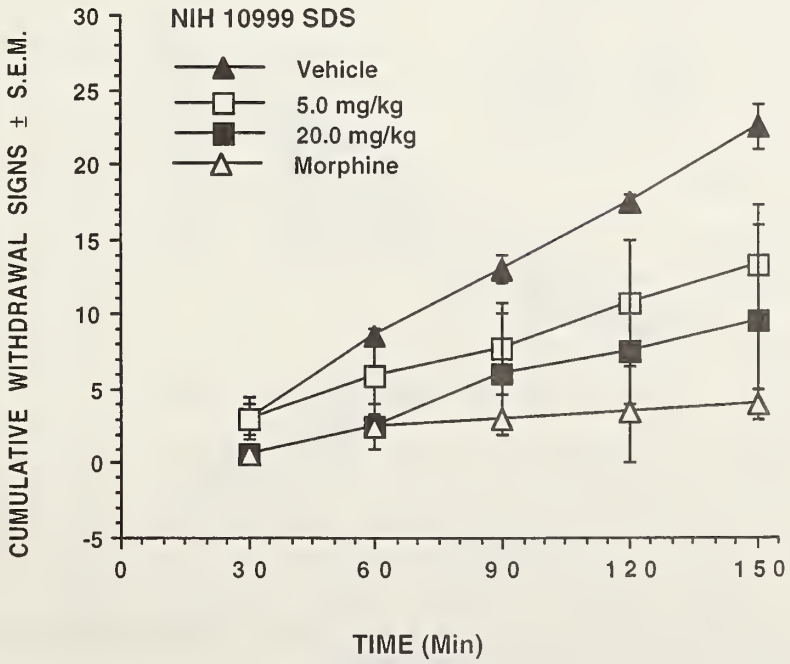
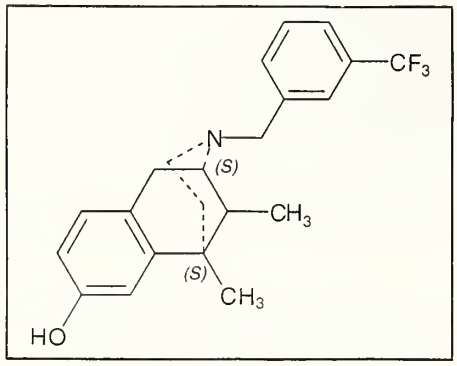


Fig NIH 10999-SDS. Results of study in which single doses of NIH 10999 were substituted for morphine in morphine-dependent monkeys in withdrawal.

Comment: Apparently, there is a species difference with this drug. Nevertheless, in the monkey, mu-opioid receptor agonist properties are apparent.

NIH 11000 (+)-(1*S*,5*S*,9*S*)-5,9-Dimethyl-2'-hydroxy-2-(3-trifluoromethylbenzyl)-6,7- benzomorphan hydrochloride



MOUSE DATA - ED50 OR AD50
(95 % C.L.) (mg/kg or % change)
1) TF – Inactive at 1, 10 and 30[a]
2) TF vs. M – Inactive at 1, 10 and 30[a]
3) PPQ – 0% at 1, 11% at 10 and 9% at 30[a]
4) HP – Inactive at 1, 10 and 30[a]

[a] Vehicle was 8% hydroxypropyl-β-cyclodextrin in water.

MONKEY DATA
(SDS)

NIH 11000 neither substituted for morphine nor exacerbated withdrawal at doses of 4 and 16 mg/kg (see Fig NIH 11000-SDS.).

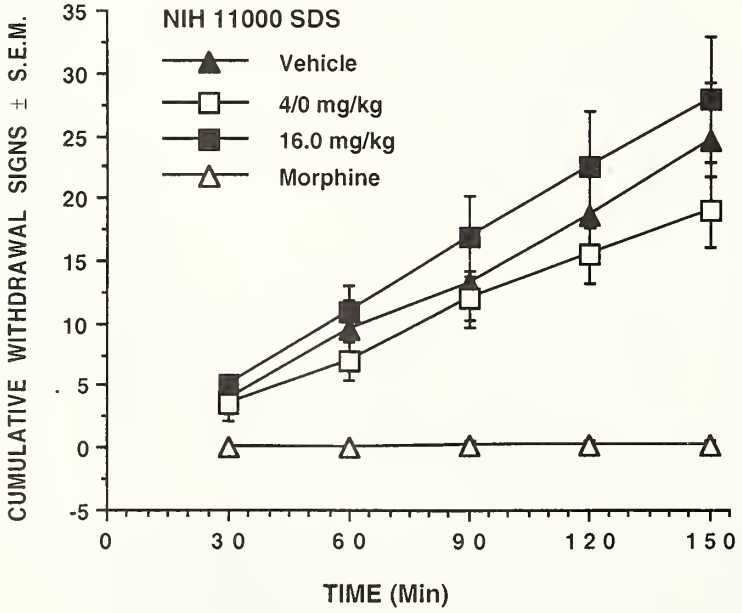
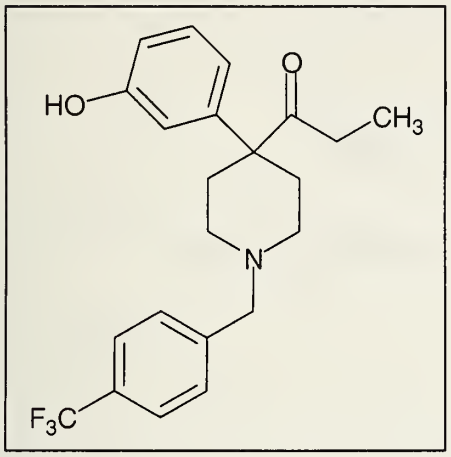


Fig NIH 11000-SDS. Results of study in which single doses of NIH 11000 were substituted for morphine in morphine-dependent monkeys in withdrawal.

Comment: The results in the mice and monkeys do not portend significant mu-opioid receptor activity in these monkeys.

NIH 11001 4-(3-Hydroxyphenyl)-4-(1-oxopropyl)-1-(4-trifluoromethylbenzyl)piperidine hydrochloride



MOUSE DATA - ED50 OR AD50
(95 % C.L.) (mg/kg or % change)

1) TF – 0% at 1 and 10, 4% at 30[a]
2) TF vs. M – Inactive at 1, 10 and 30[a]
3) PPQ – 0% at 1, 6% at 10 and 17% at 30[a]
4) HP – Inactive at 1, 10 and 30[a]

[a]Vehicle was 5% hydroxypropyl-β-cyclodextrin in water.

MONKEY DATA
(SDS)

Limited supplies permitted the testing of only 1 monkey at each dose, namely, 4 and 16 mg/kg (see Fig NIH 11001-SDS). Some eyelid ptosis was noted at the high dose. The results are not indicative of opioid activity.

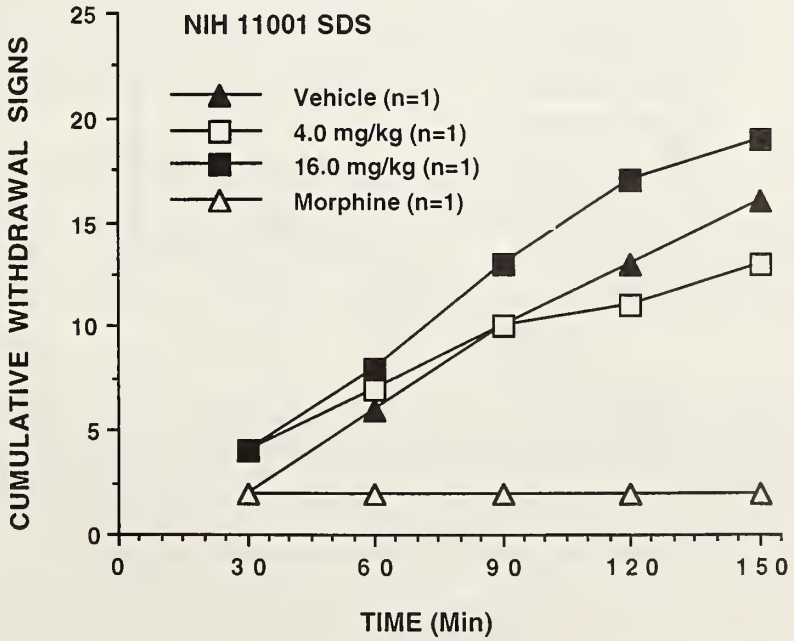
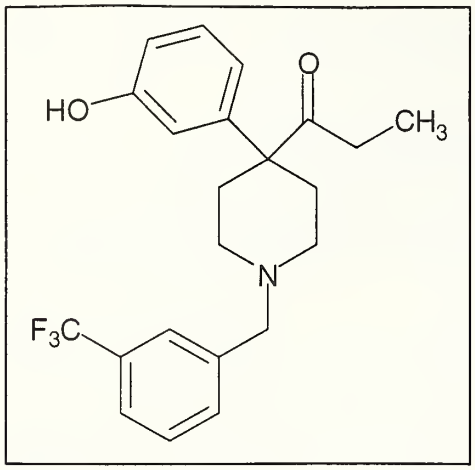


Fig NIH 11001-SDS. Results of study in which single doses of NIH 11001 were substituted for morphine in morphine-dependent monkeys in withdrawal.

Comment: NIH 11001 is devoid of mu-opioid receptor activity in these tests.

NIH 11002 4-(3-Hydroxyphenyl)-4-(1-oxopropyl)-1-(3-trifluoromethylbenzyl)piperidine hydrochloride



MOUSE DATA - ED50 OR AD50
(95 % C.L.) (mg/kg or % change)

1) TF – Inactive at 1, 10 and 30[a]
2) TF vs. M – Inactive at 1, 10 and 30[a]
3) PPQ – Inactive at 1, 10 and 30[a]
4) HP – Inactive at 1, 10 and 30[a]

[a]Vehicle was 5% hydroxypropyl-β-cyclodextrin in water.

MONKEY DATA
(SDS)

Based on limited testing due to exhausted drug supply, NIH 11002 had little effect in the morphine-dependent monkey (see Fig NIH 11002-SDS). It did not substitute for morphine, attenuate or exacerbate withdrawal. Vehicle was 10% hydroxypropyl-β-cyclodextrin in water.

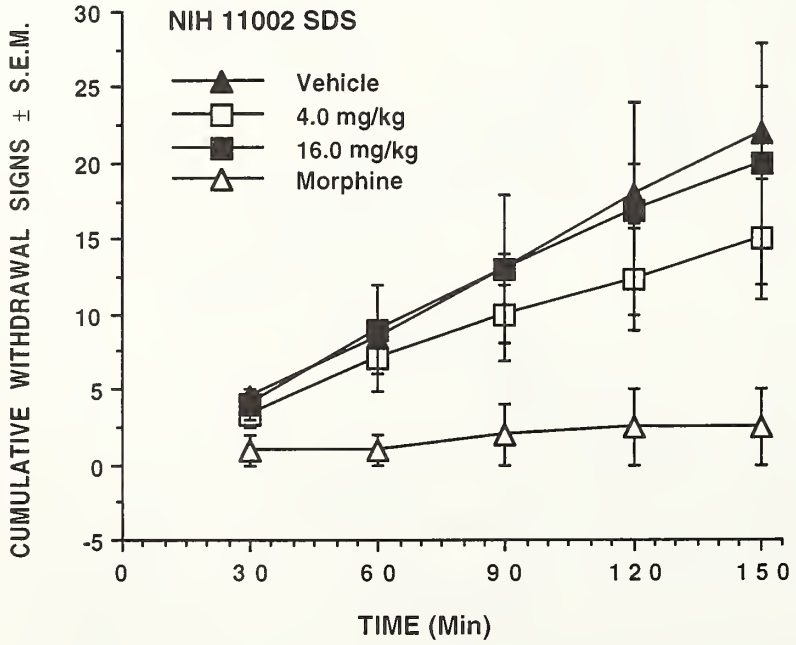


Fig NIH 11002-SDS. Results of study in which single doses of NIH 11002 were substituted for morphine in morphine-dependent monkeys in withdrawal.

Comment: It is unlikely that NIH 11002 has opioid properties in these assays.

NIH 11008 *S*-(+)-Mecamylamine hydrochloride
(*S*)-(+)-N,2,3,3-Tetramethyl-bicyclo[2.2.1]heptan-2-amine hydrochloride

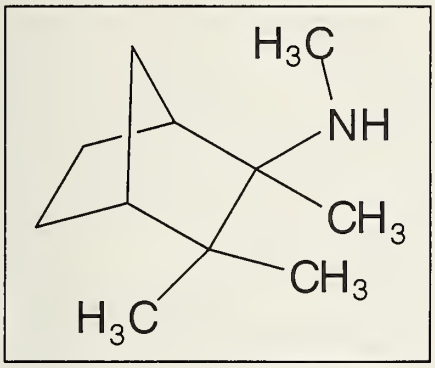


Table 1. Antinociceptive effects of NIH 11008 using the tail flick (TF) and phenylquinone (PPQ) assays and its interactions with the nicotinic cholinergic and opioid systems.

Assay	Route of Administration	Pretreatment time (min)	ED50 (mg/kg or % change)	Remarks
TF[a]	s.c.	20	Inactive at 1, 10 and 30	
	s.c.	5	Inactive at 1, 10 and 30	
	i.v.	5	Inactive at 1, 10 and 30	All 6 died at 30
PPQ[b, c]	s.c.	20	11.40 (4.64-27.98)	

[a]NIH 11008 reduced the control reaction time in many mice with the tail-flick test.
[b]Mecamylamine, (s.c.) inactive at 1 and 10 mg/kg vs ED80 of NIH 11008 in the PPQ test.
[c]Naloxone(s.c.) inactive at 1 and 10 mg/kg vs ED80 of NIH 11008 in the PPQ test.

Table 2. Effects of NIH 11008 in the ED80 of nicotine[a] in the tail-flick test.

Administered	Pretreatment Time	AD50 (mg/kg or % change)
s.c.	20 min	0.03 (0.01 - 0.07)

[a]Nicotine was given s.c. 5 min before testing.

Comment: By various routes of administration and different pretreatment times, NIH 11008 was inactive in the tail-flick test. Modest activity was detected in the PPQ test which was not blocked by either mecamylamine or naloxone. Apparently, NIH 11008 antinociception did not involve either the nicotinic cholinergic or the opioid agonist mechanisms. Nevertheless, NIH 11008 is a potent nicotine antagonist.

NIH 11009 *R*-(-)-Mecamylamine hydrochloride
(*R*)-(-)-N,2,3,3-Tetramethyl-bicyclo[2.2.1]heptan-2-amine hydrochloride

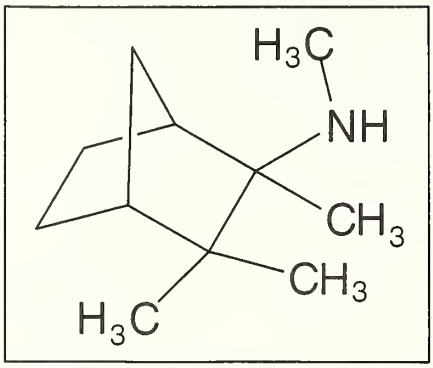


Table 1. Antinociceptive effects of NIH 11009 using the tail flick (TF) and phenylquinone (PPQ) assays and, its interactions with the nicotinic cholinergic and opioid systems.

Assay	Route of Administration	Pretreatment time (min)	ED50 (mg/kg or change)	Remarks
TF[a]	s.c.	20	Inactive at 1, 10 and 30	Immobile, eyelid ptosis and tremors
	s.c.	5	Inactive at 1, 10 and 30	
	i.v.	5	Inactive at 1 and 10	Four of 6 died at 10[b]
New Data	s.c.	20	Inactive at 0.01, 0.05 and 0.1	
PPQ [c,d]	s.c.	20	9.47 (4.45-20.24)	

[a]NIH 11009 reduced the control reaction time in the tail-flick test.
[b]Mecamylamine(10 mg/kg, s.c.) pretreatment: Four of 6 died after NIH 11009 (10 mg/kg, i.v.).
[c]Mecamylamine(1 and 10 mg/kg, s.c.) pretreatment: Inactive versus ED80 of NIH 11009 in the PPQ test.

[d]Naloxone (1 and 10 mg/kg, s.c.) pretreatment: Inactive versus ED80 of NIH 11009 in the PPQ test.

Table 2. Effects of NIH 11009 vs the ED80 of nicotine[a] in the tail-flick test.

Administered	Pretreatment Time (Min)	AD_{50} (mg/kg or % change)
s.c.	20	0.12 (0.04 – 0.32)

[a]Nicotine was given s.c. 5 min before testing.

Comment: NIH 11009 appeared devoid of antinociceptive activity in the tail-flick test. Activity in the PPQ test seemed impervious to pretreatment with either mecamylamine or naloxone suggesting little, if any, agonist interactions with either the cholinergic nicotinic or opioid system. However, NIH 11009 was very active vs nicotine. This compound is a nicotine antagonist.

NIH 11010 (±)-Mecamylamine hydrochloride
(±)-N,2,3,3-Tetramethyl-bicyclo[2.2.1]heptan-2-amine hydrochloride

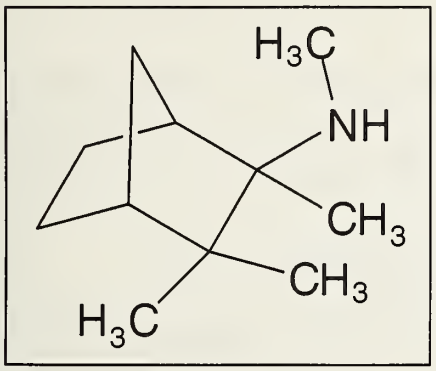


Table 1. Antinociceptive effects of NIH 11010 using the tail flick (TF) and phenylquinone (PPQ) assays and, its interactions with the nicotinic cholinergic and opioid systems.

Assay	Route of Administration	Pretreatment time (min)	ED50 (mg/kg or % change)	Remarks
TF[a]	s.c.	20	Inactive at 1 and 10	Eyelid ptosis, tremors, and rapid respiration
	s.c.	5	Inactive at 1, 10 and 30	
	i.v.	5	Inactive at 1, 10 and 30[a]	All died at 30. At 10 mg/kg, 2/6 died.
New Data	s..c.	20	Inactive at 0.01, 0.05 and 0.1	
PPQ[b,c]	s.c.	20	4.23 (2.09 - 8.55)	

[a]NIH 11010 reduced the control reaction time in the tail-flick test.
[b]Mecamylamine(1 and 10 mg/kg, s.c.) pretreatment: Inactive versus the ED80 of CPDD NIH 11010.
[c]Naloxone (1 and 10 mg/kg, s.c.) pretreatment: Inactive versus the ED80 of NIH 11010 in the PPQ test.

Table 2. Effect of NIH 11010 vs the ED80 of nicotine[a] in the tail-flick test.

Administered	Pretreatment Time	AD_{50} (mg/kg or % change)
s.c.	20 min	0.36 (0.18 - 0.71)

[a]Nicotine was given s.c. 5 min before testing.

Comment: NIH 11010 was inactive, antinociceptively, in the tail-flick test. Activity in the PPQ test was resistant to blockade by either a cholinergic nicotinic blocker or an opioid antagonist. However, the drug displays nicotine antagonist properties.

ACKNOWLEDGMENTS

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AFFILIATION: Department of Pharmacology and Toxicology, School of Medicine, Virginia Commonwealth University, Richmond, VA 23298-0613

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SUBJECT INDEX

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