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Opioid Receptors: Some Perspectives from Early Studies of Their Role in Normal Physiology, Stress Responsivity, and in Specific Addictive Diseases*

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The early history of research on the possible existence of specific opioid receptors and on developing a new form of pharmacotherapy for the treatment of heroin addiction in New York City, from 1960–1973, along with the special relationships between two leading scientists conducting these research efforts, Dr. Eric Simon and Dr. Vincent P. Dole Jr., are presented in a historical perspective. The linkage of these early efforts and the subsequent identification and the elucidation of the effects of exogenous opiates acting at specific opiate receptors in human physiology, including some findings from perspective studies of heroin addicts at time of entry to and during methadone maintenance treatment, are presented in the context of the important clues which thereby were provided concerning the possible roles of the endogenous opioids in normal mammalian physiology. From many of these early clinical research findings and studies in animal models, the hypothesis that the endogenous opioids system may play an important role in stress responsivity was formulated along with the related hypothesis, first presented in the early 1970s, that an atypical responsivity to stress and stressors might be involved in the acquisition and persistence of, and relapse to specific addictive diseases, including heroin addiction, cocaine dependency and alcoholism. More recent studies of the possible involvement of the specific opioid receptors in these three addictive diseases—heroin addiction, cocaine addiction and alcoholism—from our laboratory are discussed in a historical perspective of the development of these ideas from the early research findings of not only Dr. Eric Simon, but his numerous colleagues in opioid research in the United States and throughout the world.

KEY WORDS: Opioid receptors; stress responsivity; addictive disease.

Early History: Research on Treatment of Heroin Addiction and Existence of Opioid Receptors 1960–1973 in New York City

There are only a few times in one's academic research career when there is the opportunity and privilege of writing a paper in honor of a scientist who has been not only a wonderful contributor to a particular field of research but also, as is certainly true in this case, who has been an enormously supportive colleague and, who early on had the courage to support a concept and then the reality which was then foreign to most, and unacceptable, unfortunately, to many, that is, the long term pharmacotherapy of opiate addiction. A few years before I had the privilege of meeting Dr. Eric Simon, he became a close friend, and also a working colleague, of my original mentor here at The Rockefeller University (then the Rockefeller Institute for Medical Research) in the field of research which was to become the major focus of my own scientific career, Professor Vincent P. Dole, Jr.

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The late Dr. Lewis Thomas had recruited both Dr. Dole and Dr. Simon to serve on the "Working Group on Narcotic Addiction of the Health Research Council of the City of New York." Dr. Eric Simon was to serve as Secretary of that group from 1960 to 1964, and when Dr. Thomas took a sabbatical year away from New York, Dr. Dole was to become the Chairman of that Working Group. I was in medical school at the Columbia University College of Physicians and Surgeons at that time, and later an intern and resident in internal medicine at the New York Hospital-Cornell Medical Center. I had not yet had the privilege of meeting either Dr. Dole (whom I first met in the early autumn of 1963) or Dr. Eric Simon (whom I met shortly thereafter). However, it is my understanding that the Health Research Council committee provided an extraordinarily important intellectual milieu, in part, directed toward marshaling resources for what was defined as the single major unaddressed health care problem in New York at that time, that is, heroin addiction.

At the same time, this committee through its deliberations, created an environment, with, I suspect, a profound and exciting intellectual ferment which had pivotal influence on the careers of both Dr. Simon, and Dr. Dole, who subsequently was to shift his entire laboratory effort (as well as ultimately his laboratory name) from studies related to physiology and metabolism, with emphasis on lipid metabolism, obesity, electrolyte balance and hypertension, to clinical, and later related laboratory, research focused on the problem of heroin addiction, and later, in the mid 1970s, to laboratory studies on the problems of alcoholism. I think it is quite probable that this same ferment contributed to a refocusing of the biochemical and early molecular biological work of Dr. Eric Simon, since it was in 1963 that his first paper on these topics, "Inhibition of RNase synthesis of E. coli by the narcotic drug levorphanol" appeared in *Nature*, followed by a later paper "Inhibition of bacterial growth by drugs of the morphine series" published in *Science* in 1964, and then, followed by two papers written by Simon and D. Van Praag on the selective inhibition of RNase synthesis in E. coli by opioids, which appeared in the *Proceedings of the National Academy of Sciences* (1–4).

While a resident in internal medicine, I was allowed to spend a research elective beginning in early 1964 with Professor Vincent Dole at the then Rockefeller Institute for Medical Research and to participate in the very first studies led by Dr. Dole on the possible use of an opioid in the long term management of opiate addiction (5–7). The late Dr. Marie Nyswander, a psychiatrist who had vast and frustrating experiences in attempts to manage heroin addiction by diverse drug-free approaches, including individual and group psychiatric and psychological care, and behavioral modification, work conducted in part at the USPHS facility in Lexington, Kentucky, as well as at the Bellevue Hospital in New York, and in various communities of the inner city of New York City, also joined Professor Dole at this time. Dr. Nyswander had written a book in 1956, *The Addict as Patient*, which had attracted the attention of Dr. Dole because of its eloquent presentation of the medical dilemma and the societal tragedy of the enormous number of lost lives of unsuccessfully treated heroin addicts, who had been managed either by prison, alone, or by short-term pharmacological intervention followed by attempts at drug-free management (8).

In our earliest work in 1964 conducted at The Rockefeller Hospital, we elected to study a synthetic opioid, methadone, which had been used to a limited extent at heroin detoxification centers, including Bellevue and Lexington, and which, based on clinical observations, had some indications of a longer acting pharmacokinetics profile than morphine or heroin (9). At that time there were no sensitive and specific analytical techniques for determining plasma or even urine levels of any opiate; therefore, meticulous clinical observations carried out in the setting of the basic clinical research unit of the Rockefeller Hospital were necessary to determine the pharmacokinetic properties of methadone. The early clinical research studies confirmed the fact that methadone was orally effective and, as suspected, had a much longer duration of action than morphine, heroin or any other available opiate. These early studies also revealed that a single daily oral dose of methadone would prevent the signs and symptoms of the opiate abstinence syndrome (that is "withdrawal" symptoms) and, also of great importance, would prevent "drug hunger" or drug craving throughout the 24-hour dosing interval (5,9).

Further clinical research studies, each four weeks in duration and utilizing a double-blinded, random order, Latin square design protocol, which were conducted at the Rockefeller Hospital, showed that the superimposition of a short-acting opiate, such as heroin, morphine or dihydromorphone, or the long-acting opioid, methadone, or saline, each delivered as a single, bolus intravenous dose against a daily background of oral administration of a treatment dose (in this case, 80 mg/day) of methadone resulted in no subjective or objective signs or symptoms of any narcotic effect, either detected by the research subject (by then, an early "methadone maintained" patient in treatment from eight to 12 weeks) or by the clinical investigators (5). The only sign detected by the research subjects-patients was

that a "pins and needles" sensation following the intravenous injection of morphine, which, however, was not followed by any "rush" or euphoric "high," the absence of which was remarkable to the addict, well-versed in the special effects of morphine when administered intravenously (5).

The lack of any observable subjective or objective signs and symptoms following the superimposition of a short- or long-acting opioid against the background of daily methadone treatment was of great theoretical and practical importance. These findings were of theoretical importance, because they revealed one of the mechanisms which has proven to be so important for effective methadone maintenance, that is, through the phenomena of tolerance developed to the high doses of methadone, as used in effective treatment, (60–120 mg/day stabilized doses, in most subjects, that are achieved after slow escalation from a starting dose of 20–40 mg), there is also cross-tolerance to other superimposed, short-acting opioids, all now known to be primarily mu opioid receptor preferring ligands (in most cases, heroin, though in some cases, morphine, meperidine, dihydromorphone, or more recently, fentanyl and its congeners). Also, of practical importance was the fact that no signs and symptoms of narcotic overdose occurred when short-acting opioids were superimposed, that is, no respiratory depression or other problems, which would have precluded chronic use of high dose of methadone in treatment (5).

Of both theoretical interest and practical importance were the implications of these findings, in the context of classical conditioning theory. It was assumed (and now reproven for over 30 years) that most heroin addicts entering, inducted into and gradually stabilized on high dose methadone maintenance treatment, experience no euphoric or narcotic-like effects when an appropriate slow induction and methadone dose escalation is conducted. However, many, or even most, heroin addicts during very early treatment would still like to experience an opiate effect and thus, try to achieve the euphoria, or to become "high," by the superimposition of a short-acting opiate, primarily heroin. As was found in these early cross-tolerance studies, no "reward," that is, no reinforcing effects were forthcoming following intravenous administration of heroin against a background of methadone treatment (5). We predicted that probably increasing doses of heroin would be tried, up to the economic limits of procurement, and thus, in our early studies we titrated the dose of heroin which would be required to overcome the cross-tolerance provided by moderate to high dose methadone maintenance treatment (5). We found no narcotic-like effects were experienced or observed by the patient or clinical observer until very high doses of heroin (then exceeding a $200 street value) were superimposed against the background of steady dose methadone treatment (5). Thus, in former heroin addicts during early treatment and "on the street," when no "rewarding" or reinforcing effects were forthcoming after self-administration of affordable amounts of heroin, deconditioning, or extinction, phenomena would evolve, with a decreasing behavior of heroin self-administration. The former addict now in methadone treatment might reduce or cease all heroin use even before completion of what would be a classical extinction protocol, or alternatively, in some cases, exercise the option of leaving methadone maintenance treatment to return to their lifestyle, and problems, of the heroin addict on the street (5).

What was found in the 1960s in our earliest research, and which has been subsequently found repeatedly in the 1970s, 1980s and 1990s, is that in effective programs, which combine use of proper doses of methadone, following appropriate and slow induction, with on site counseling, provided as needed and appropriate, along with access to medical and psychiatric care, preferably also on site, may yield enormously successful results, with over an 80% retention in treatment for one year or more, and after six months of stabilization of methadone, a reduction of illicit opiate use to less than 15% (6,7,10–16). In addition, criminal behavior is markedly reduced, as documented by decreases in arrest and imprisonment, and productivity is increased with and restoration to a normal lifestyle achieved in the majority of patients, as shown by increasing numbers of patients in gainful employment, homemaking and educational pursuits (6,7,10–14).

It has also been found that even when the most limited services are provided, when adequate doses of methadone are used (60 to 120 mg per day in most patients), 45% to 55% of patients will stay in treatment and exhibit a significantly reduced illicit use of heroin (15,16). However, other problems must be addressed, including other continuing chemical dependencies which have always been very common in heroin addicts. Now, in some areas, there is an over 80% prevalence of concomitant cocaine dependency, and for many years, since the inception of our studies in 1964, a 25% to 50% concomitant alcohol abuse problem has pertained in many regions; these problems will continue at a higher prevalence in some programs, as will other types of co-morbidity and profound behavioral disruptions (6,7,10, 12,15,16). Therefore, clearly effective treatment must combine both pharmacotherapy and other adjunctive treatments as needed.

Dr. Eric Simon was enormously supportive of the early research on pharmacotherapies, and a helpful and strong ally of especially Dr. Dole and Dr. Nyswander, and the rest of our research and treatment team, as attempts were made to introduce this pharmacotherapy more broadly, initially into various hospitals in the City of New York.

In further research, using the increasingly available sophisticated analytical techniques to quantitate plasma and urine levels of medications, it became possible to determine the disposition of methadone, including metabolism and pharmacokinetics. Early studies, conducted primarily by the groups of Dr. C. Inturrisi at New York Hospital-Cornell Medical Center and my Laboratory of the Biology of Addictive Diseases at The Rockefeller University, proved that methadone as used in chronic treatment in humans, has a long-acting pharmacokinetic profile, with a 24-hour apparent terminal plasma half-life in humans (17–29). Methadone was unique amongst opioids which had been introduced by that time, and had properties which were soon shown to be shared only by its longer acting congener, l-alpha-acetylmethadol (LAAM). Thus, further rationale for use of this medication in treatment of opiate addiction was provided. Only recently has the very high mu opioid receptor selectivity of this synthetic ligand been documented by research in which cloned mu-specific opioid receptors are transfected into appropriate cells for studies of cell surface ligand-receptor binding and also signal transduction activity. However, even early on in 1963–64, we had hypothesized that methadone acted primarily or solely at the same "site of action" as heroin in its primary effects, thus, providing the rationale for its use in the pharmacotherapy of opiate addiction.

From the beginning of our work at The Rockefeller University in the winter of 1963/64 and thus, the beginning of our conceptualization of the goals for pharmacotherapy for heroin addiction (primarily to prevent opiate withdrawal symptoms and reduce or eliminate "drug hunger" or craving and drug seeking behavior), and also of the desirable properties of a pharmacotherapeutic agent (an orally effective agent, with slow onset of action, long duration of action, slow offset of action, to prevent any reinforcing effects of the medication and to allow sustained, steady state action over a full-dosing interval, preferably of at least a 24 hours, that is, a one day duration of action), we also developed a definite rationale for treatment. The rationale was to target a treatment agent at a specific receptor site, or some other site of action known to be perturbed by chronic use of the short-acting drug of abuse such as heroin (11).

In this context, from the very beginning of our work, Dr. Dole always discussed the presence of "specific opiate receptors," as if they indeed had been completely defined. Over the next few years, several groups in the United States and Europe all began to report studies in which the concept of "specific opiate receptors" was introduced, and based primarily on pharmacological observations, the concept of multiple opiate receptor types was also introduced. However, no specific documentation of the existence of any specific opiate receptor was to be forthcoming for a few years.

Working with a graduate student, now Dr. N. Ingoglia, Dr. Dole developed a research protocol in the late 1960s to attempt to delineate the presence of specific opiate receptors, a protocol in which the possibility of differential stereoselective binding by a specific opiate receptor of an active versus inactive enantiomer of a specific opioid agonist, was studied, in this case, using methadone itself as the ligand (30,31). Since no radioactive compounds were available for such studies, the separate enantiomers of methadone were tritiated by Dole and colleagues at the Rockefeller University for their own use in these studies. However, the specific activity of these compounds was extremely low and thus, although combined specific and nonspecific binding could be measured (which led later to studies of the long term persistence of such binding in specific tissues), it was not possible to delineate specific binding alone (30,32). Based on these various studies, however, Dole was able to predict with remarkable accuracy both the density and, in part, the localization of specific opiate receptors in brain tissue and also to predict their presence at peripheral sites (31). Within the next year, Dr. Avram Goldstein reported another rigorous attempt to define the presence of specific opioid receptors, again using an approach of stereoselective binding (33). However, the differences between specific and nonspecific binding remained small, with detection again limited because of low specific activity of the opioid ligands.

It was therefore in 1973, following the availability of radiolabeled stereoselective ligands with much higher specific activity, that, within an extremely short period of time, three groups, each working independently and each using the experimental approach of stereoselective binding, but using different ligands, the group of Dr. S. Snyder with Dr. C. Pert working at the Johns Hopkins University in Baltimore, the group of Dr. E. Simon working with Dr. J. Hiller at New York University in New York City, and the group of Dr. L. Terenius working in Uppsala University, Sweden, reported the presence of specific opioid receptors (34–36). These were

seminal accomplishments, upon which many other studies by numerous research groups have been subsequently based. A few of these studies from our laboratory will be discussed in this brief review.

The major role of Dr. Eric Simon in this achievement of the discovery and elucidation of specific opioid receptors was undoubtedly in part due to the exciting intellectual challenges which confronted him, along with Dr. Dole, in their first work for the New York City Health Research Council beginning in 1960. Also, the very important support which Dr. Simon gave to all of our early clinically based research work at The Rockefeller University for developing a pharmacotherapeutic agent for heroin addiction, may have served, in part, as an additional impetus to the vigorous activities of his own laboratory in aggressively studying many aspects of narcotic effects on single cells with respect to metabolism, RNA synthesis and other factors related to opioid receptor binding. All of this research contributed to his early and very important contribution of defining the existence of specific opioid receptors and his subsequent extensive work on defining the structure and activities of those receptors.

Effects of Exogenous Opiates Acting at Specific Opiate Receptors in Human Physiology (Including Some Findings from Prospective Studies of Heroin Addicts at Entry to and During Methadone Maintenance Treatment): Clues to the Roles of Endogenous Opioids in Normal Physiology

As soon as the initial studies on the possible efficacy of use of the long-acting opioid methadone in the long-term pharmacotherapy of opiate addiction were conducted at The Rockefeller University, including the studies discussed above delineating the phenomena and extent of tolerance and cross-tolerance developed during chronic methadone treatment in humans, two other lines of investigation were initiated in the late spring of 1964 (5,9). First, a replication of the initial treatment research was conducted under the leadership of Dr. Marie Nyswander, but this time, at what was then a proprietary heroin detoxification unit, The Manhattan General Hospital, (later to become the very excellent and dedicated Bernstein Institute of the Beth Israel Medical Center) (37). Secondly, studies to determine both the medical status of heroin addicts entering treatment, as well as during induction into methadone maintenance treatment and prospectively, of the medical and more subtle physiological effects of long-term methadone maintenance treatment (38–41).

The purpose of these studies was first to define in greater detail the health status profile of untreated heroin addicts entering treatment research in New York City, and then to determine the impact of treatment on both health status and physiological functions. Several health status studies had been conducted, primarily at the USPHS Hospital in Lexington, Kentucky, in various populations of primarily heroin addicts in prison. However, it was important to determine precisely the spectrum of diseases present in heroin addicts entering treatment in New York in the mid-1960s, as well as to begin to determine physiological alterations and abnormalities which had been potentially caused by chronic use of the short-acting opiate, heroin. It was also essential to determine the safety and effects of methadone as used in short- and long-term maintenance treatment. Thus, studies both of any changes in health status and in physiology during early induction treatment, but also following methadone dose escalation induction and stabilization in treatment were essential.

These first prospective studies, conducted by my group, which included a rigorous evaluation of general health status, but also physiological status, and in all entrants in methadone maintenance treatment research beginning in 1964 onward, eventually included the first 214 consecutive patients admitted to treatment research either at the Rockefeller University, or at the Manhattan General-Bernstein Institute Facility and the soon to be created other clinics primarily under the auspices of the Beth Israel Medical Center, including later, St. Luke's and Roosevelt Medical Centers affiliated at that time with Columbia University, as well as the clinics affiliated with Albert Einstein Medical Center (38–41). In addition, retrospective studies were conducted by my group of large numbers of patients admitted during the first five years of methadone maintenance treatment research and the extension of that research into the field (40). Also, special studies were conducted primarily in the in-patient General Clinical Research Center of the Rockefeller University Hospital to study in greater depths some of the physiological changes which we had observed (21,22,38–43).

In these studies it was defined that the major chronic disease present in untreated heroin addicts in the mid-1960s was chronic hepatitis, in varying degrees of severity (39,40,43). As soon as hepatitis B serological tests became available, after the delineation of the hepatitis B versus other forms of hepatitis in the late 1960s, viral hepatitis marker studies were performed in patients entering and during methadone maintenance treatment research; and sera were prospectively collected and ret-

roactively analyzed as needed. It was found that over 80% of patients entering treatment in the 1960s had been infected with the hepatitis B virus, as evidenced by presence of protective antibodies (anti-HBs); and later, when tests were available; alternatively, by presence of the non-protective, core antibodies (anti-HBc); others were still actively infected with hepatitis B virus with evidence of possible hepatitis B replication (HBs-Ag). Chronic sequela of a variety of infectious diseases, primarily of hepatitis B or endocarditis, were present in some patients. Concomitant alcohol abuse or alcoholism was identified in 25 to 50%, with concomitant use of a variety of other drugs including primarily, at that time, barbiturates, amphetamines, or then only rarely, cocaine (39,40,43).

Of great interest with respect to later determining the role of opioid receptors and their endogenous ligands in normal physiology, however, were the many abnormalities of normal physiology found in untreated heroin addicts, many of which persisted during the early induction and stabilization of methadone maintenance treatment. These identified alterations in normal physiology included a disruption of the very important stress responsive hypothalamic-pituitary-adrenal axis system, findings which were then made in 1964–73 and now continue to be actively pursued by our laboratory (38,40,41,43). These findings led to our early 1970s hypothesis that an atypical responsivity to stressors may contribute to the acquisition and persistence of drug craving, drug seeking behavior and addiction, and to relapse to drug use after periods of abstinence.

Also, disruptions of hypothalamic-pituitary-gonadal axis were observed in our early studies and in studies of other groups (39,40,42–44). Most notably, when the first women were entered into methadone maintenance treatment research, it was found that many had experienced very irregular menses for many years during heroin addiction or had developed secondary amenorrhea, along with relatively reduced fertility. Although these abnormalities had for years been attributed to the stigmata of chronic sexually transmitted diseases, such as syphilis and gonorrhea, other studies determined that most of the positive tests for syphilis were indeed biologic false positive tests due to abnormalities in immune function (39,40,43). Of great importance, it was found early on that female patients, following induction and stabilization in methadone maintenance treatment, experienced a return to normal menstrual cycling, and desired pregnancies ensued. Further studies by several groups showed that the short-acting heroin opiates such as heroin, in humans, as well as in appropriate animal models (not to be discussed here), reduced especially the pulsatile luteinizing hormone (LH) release with subsequent anovalatory cycles or frank secondary amenorrhea (43). Also, in males, testosterone levels were found to be reduced during cycles of heroin addiction (43).

However, following stabilization on methadone treatment, it was shown both by our group and by others that LH levels return to normal, and that testosterone and estrogen levels become normal, and pulsatile release of LH return to normal with normal preovulatory surges of LH. These findings, which have been subsequently built upon by many groups including many significant clinical research studies by the groups of Mendelson and Mello at Harvard University, and of Cicero at Washington University and also Santen at Pennsylvania State University, have provided documentation that endogenous opioid receptors and their endogenous opioid peptide ligands are involved in the normal physiological regulation of LH release and especially of pulsatile LH release (43). These studies in each case have been conducted, in part, by use of a specific opioid antagonists, primarily naloxone and naltrexone, which became available for use in human studies by the mid 1970s.

Numerous immune function abnormalities were found in early studies of heroin addicts entering methadone maintenance treatment research, long before the advent of AIDS around 1978 (39,40,43). Subsequent clinical research studies in long-term methadone maintained patients have shown that normalization of immune function occurs during chronic moderate to high dose treatment, possibly due, in part, to normalization of neuroendocrine function (45). However, numerous laboratory, as well as clinical research studies from our laboratory and others have provided increasing evidence that opiates, other drugs of abuse and the endogenous opioids may modulate or alter the immune function.

Another aspect of normal physiology which was found to be deranged in the studies of heroin addiction, and also notably during the first few years of methadone maintenance treatment, was gastrointestinal function (38,40,43,46,47). Abdominal cramps and diarrhea have long been recognized as a major sign of opiate withdrawal. It was quite clear that when short-acting opiates were used on a chronic basis, gastrointestinal hypomotility occurs and persists with the end result of constipation (38,40,43). Natural opiates have been used for thousands of years to manage diarrheal conditions in humans. In our prospective studies of patients entering and in methadone maintenance treatment, constipation was a major problem, and one which persisted for up to three years or more in the long-term prospective studies in over 20% of the subjects studied (40,43,46,47). However, it also was found, contrary to previous published

works and textbooks of pharmacology, that tolerance ultimately would develop to the constipating effects of opiates, at least with chronic use of long-acting opioids, in this case, methadone, when used on a stabilized, steady dose basis.

Initially it was presumed that these opiate effects on gastrointestinal motility were mediated through the central nervous system control mechanisms. However, several studies from our laboratory, as well as from others, confirmed that, in addition to central nervous system mediation of gastrointestinal motility, there is very significant regulation of gastrointestinal motility within the intestinal wall itself at all levels through various neurotransmitter and neuropeptide systems. Very specifically, our group found that an orally administered opioid antagonist, which has essentially no systemic bioavailability after oral administration (naloxone) can modulate, and partially or completely reverse opiate induced constipation (46,48–52). In studies conducted in animal models we have also found that there is opiate receptor-type selectivity of this gastrointestinal response to any orally administered opioid agonist, as well as opioid antagonist. These studies have shown that in the guinea pig (and probably in man), but not in the rat, kappa opioid receptor directed ligands can slow gastrointestinal motility and also that kappa opioid receptors are present in abundance (50,51). In more recent molecular biological studies, we have shown that gene expression for preproenkephalin, yielding the propeptide proenkephalin which in turn yields multiple-delta and mu opioid receptor directed peptides, is abundant throughout the gastrointestinal tract of the guinea pig, with especially high levels of gene expression as measured by classical solution hybridization protection assays in the small intestine (53,54). More recently, the guinea pig dynorphin gene has been cloned by our laboratory (55). Using riboprobes derived from this cloned gene the cDNA of this cloned gene, we have studied dynorphin gene expression in the guinea pig gastrointestinal tract, we have found measurable amounts of dynorphin gene expression, as determined quantitatively by our recently modified solution hybridization RNase protection assay throughout the gastrointestinal tract wall (56). Very high mRNA levels of this dynorphin gene have been found in the large intestine and rectum of the guinea pig (56). Further work on the relationships of the mu, kappa and delta opioid receptors and their various endogenous opioid peptide ligands, both in normal gastrointestinal physiology, as well as the possible role of the endogenous opioid system in such gastrointestinal disorders as idiopathic chronic constipation continue to be under study in our laboratory (48,49). Of particular interest for our work in addictive diseases continues to be the impact of short-acting versus long-acting opioids in causing persistent abnormalities of gastrointestinal motility, and the possible management of these disorders especially in methadone maintenance treatment patients and also chronic pain patients by use of a non-systematically bioavailable specific opioid antagonist (46).

Role of the Endogenous Opioid System in Stress Responsivity: Some Findings from Clinical Research and Studies in Animal Models

Very early and preliminary studies from the USPHS resource in Lexington, KY have suggested that the urinary excretion of glucocorticoids might be abnormal in the setting of heroin addiction (57). Since the beginning of our prospective studies initiated in 1964, assessment of endocrine and neuroendocrine function was included as part of the special studies (38,40,41, 43,58,59). In these early studies, we conducted both repeated measured studies of basal function of the hypothalamic-pituitary-adrenal axis, using the then-available assays which were quite limited, primarily measurements of Porter-Silber chromogens in 24-hour urine collections; these chromogens reflect glucocorticoid synthesis by the adrenal cortex. These studies were carried out in a repeated manner in individual subjects entering methadone maintenance treatment for heroin addiction and during slow incremental increases of methadone daily oral doses up to full treatment doses (then and optimally now, between 60 and 120 mg/day) and with further repeated measures during stabilized steady-state high-dose treatment. We also had the opportunity to carry out studies in heroin addicts in early withdrawal, as well as, to a limited extent, methadone maintenance patients undergoing voluntary dose reduction and elimination.

In addition to basal studies, we also conducted metyrapone test studies, an early clinical neuroendocrinology test of "hypothalamic-pituitary reserve." Metyrapone acts by blocking the final 11 β hydroxylation step of cortisol synthesis in the adrenal cortex. In the early form of this test, repeated doses of metyrapone were administered every four hours for 24 hours; urines were collected for 24 hours for three days. The test results are determined by the extent of enhancement in the Porter-Silber chromogens which reflect the amounts of precursors of cortisol in the 24 urine collections during the second and third day following metyrapone administration. Since cortisol acts in a negative feedback mode at the hypothalamic-pituitary sites to reduce levels of trophic hormones (none of which at that time could be

directly measured in peripheral blood), one expects to see a significant increase in these trophic hormones when normal negative feedback control by cortisol is abruptly disrupted. Thus, normally one expects to see a two-fold or greater increase in excretion of the Porter-Silber chromogens, reflecting accumulation of precursors of cortisol synthesis which are then excreted in urine. What was found in these early studies was an apparent inadequate "hypothalamic-pituitary reserve," that is a decreased responsivity to this chemically-induced stressor during heroin addiction. Similar findings were made during the first two months of methadone treatment, a time when the methadone dose is being steadily increased and tolerance, as well as general adaptation to increasing doses of this synthetic opioid, are being established (38,40,41,44). However, in these early studies, in which repeated metyrapone tests were conducted in the same individuals at time of entry and induction into methadone maintenance treatment, we also found normalization of this important stress-responsive axis following three months or more of steady dose methadone maintenance treatment, with normal increases of Porter-Silber chromogens measured in urines.

With the advent of increasingly sensitive and specific assays for directly measuring first, cortisol in peripheral blood, and subsequently, measuring both ACTH and, later, beta endorphin in peripheral blood, we were able to replicate and extend these early studies. Again, we found abnormalities in both baseline studies in heroin addicts and during the early phase of methadone treatment induction. However, we found during steady moderate to high dose methadone maintenance treatment, normal levels of beta endorphin, ACTH and cortisol pertained, and also, of considerable importance, that normal circadian and normal diurnal variation in the levels of these hormones, which is disrupted during cycles of heroin addiction became normal during chronic methadone maintenance treatment (60–62). Also, we conducted further studies of stress-responsivity using metyrapone in a modified test in which a single oral dose of metyrapone is administered and blood specimens are obtained over the next eight hours. In this modified test one sees an abrupt, highly significant reduction in peripheral plasma cortisol levels within the first hour, followed by a sharp increase in plasma levels of ACTH and beta endorphin, again, reflecting a cut-off of the normal negative feedback modulation of release of these peptides, by this time known to both come from the same opioid peptide gene precursor, proopiomelanocortin from the anterior pituitary.

In these studies we determined, again, that metyrapone testing is abnormal during cycles of heroin addiction and during early methadone maintenance treatment, but becomes normalized after three months of chronic steady dose methadone maintenance treatment (11, 63,64). It was during these studies that we made the very provocative findings of the abnormal hyper-responsivity to this chemically-induced stressor in illicit opiate-free and medication-free former heroin addicts. This hyper-responsivity was of considerable interest since during heroin addiction, one finds a hypo-responsivity to metyrapone challenge, and also, since following dose reduction and elimination (detoxification) from long-term methadone treatment, one sees a very high rate of relapse, exceeding 80% in essentially all carefully conducted, long-term follow-up studies; relapse to heroin use would convert a hyper-responsive state to a hypo-responsive state (6,7,10,11). Since spontaneous excessive activity of the stress responsive axis in other settings has been associated both with anxiety states, which are very common in untreated heroin addicts, as well as in some types of depression, we postulated that although baseline unchallenged levels of hormones of the stress responsive hypothalamic pituitary adrenal axis were found to be normal in opioid-free former heroin addicts, this hyper-responsivity to metyrapone challenge might be either drug-induced (or possibly existing a priori, even before any acquisition of drug abuse on a genetic or environmentally-induced basis). We also hypothesized that this atypical responsivity to stressors may contribute to the acquisition and persistence of addictive diseases, as well as the relapse to opiate addiction, following detoxification and restoration of an opioid abstinent state.

We at first hypothesized by 1972 that an atypical stress-responsivity might be involved in opiate addiction, and the work of the mid-1980s further supported this. By the late 1980s, because of studies conducted in recently abstinent cocaine addicts in The Rockefeller Hospital, an NIH-supported GCRC, in which we had found a similar hyper-responsivity in recently abstinent long-term cocaine addicts, we extended this hypothesis to suggest that an atypical responsivity to stress or stressors may also contribute to the acquisition and persistence of cocaine addiction. Our laboratory has continued to explore the role of an atypical stress responsivity in the neurobiology of addiction in basic clinical research. Our laboratory and many others recently are pursuing parallel slides in laboratory-based studies (65–72).

In related studies conducted at that time, and we also looked at age related changes in beta endorphin levels in otherwise healthy control subjects; very provocatively we found a significant increases in plasma beta-endorphin levels in increasingly old populations of

otherwise healthy humans with the significant changes occurring at age 60 and above (73). In other related studies we had also found increasing abnormalities in gastrointestinal motility, coupled with increased beneficial responsivity to orally administered non-systemically, bioavailable antagonist naloxone in geriatric patients, suggesting an enhanced endogenous opioid level of activity with aging (74,75). Also, in related studies carried out in animal models, we found that there were increasing levels of mu and kappa opioid receptors with aging in the guinea pig colon, as determined by competitive binding assays in vitro (76). We also found that there were changes in levels of gene expression of the enkephalin gene with aging in the guinea pig model (53).

The use of opioid antagonists have obviously provided a critical tool in assessing the role of the endogenous opioid system in general, both in normal physiology and in altered physiological or pharmacological states, in both animal models of diverse kinds, as well as in human subjects, just as did careful, though opportunistic, observational clinical research in patients receiving the long-acting opioid methadone for treatment of heroin addiction, findings which essentially provided a "road map" for later determining the major actions of the endogenous opioids in humans. Use of specific opioid antagonists have provided extensive information about the normal regulation of the important stress responsive neuroendocrine axis. This work in research is, of course, limited by the selectivity of available opioid antagonists; it is also limited at the clinical research level by the availability of antagonists which may be given to human subjects. With these limitations, a great deal has been learned about the role of the endogenous opioid system by our laboratory and many others using this approach. Much of our early work on determining that gastrointestinal motility was regulated both by the intestinal wall, as well as central nervous systems sites of regulation, was made possible by the use of a differentially, systematically-bioavailable opioid antagonist following oral as contrasted to parenteral administration. The fact that naloxone has less than a 2% systemic bioavailability after oral administration both in human and in animal models allowed us to use orally administered naloxone versus intravenously administered naloxone in many different studies, leading to our elucidation of the probable role of the endogenous opioid system in normal humans, as well as in the guinea pig animal model (48–51,77,78).

Our group, as well as other groups, have also used specific opioid antagonists to study the stress-responsive systems in humans and in animal models. In earlier studies, related to our work on modulation of gastrointestinal motility by administration of opioid antagonists, we also compared the effects of bolus administration of an opioid antagonist with steady-state infusion, with respect to possible effects in neuroendocrine function (79). Building on the earliest observations by Volavka and colleagues, now associated with New York University, we found that whereas intravenous administration of naloxone caused an abrupt elevation of plasma levels of cortisol, as originally observed, and also beta-endorphin and ACTH, steady-state infusion of naloxone did not alter plasma levels of these hormones of the hypothalamic-pituitary-adrenal stress-responsive axis (80,81). We went on to compare that the hypothalamic-pituitary-adrenal axis activation by two different antagonists which may be used in humans and which are available for intravenous administration, naloxone and the longer-acting antagonist, nalmefene. Both of these antagonists are primarily directed against mu opioid receptors. However, nalmefene has greater selectivity for the kappa opioid receptor than naloxone. In a preliminary sequence of studies conducted in normal healthy volunteer humans, our findings suggested that hypothalamic-pituitary-adrenal axis may be activated not only by mu antagonists, but also by kappa opioid receptor directed antagonists (81).

The implications of all of these findings are of potentially great importance for normal human physiology: they suggest that there is normal tonic inhibition of specific components of the hypothalamic-pituitary part of the stress-responsive axis by endogenous opioids, possibly beta endorphin, as well as mu opioid receptor-selective enkephalins, and also possibly by the kappa- and mu-directed opioid dynorphin peptides. To further explore the possible differences of neuroendocrine effects of naloxone and nalmefene with some differences in opioid receptor selectivity, we have conducted a rigorous sequence of studies in which two different doses of naloxone, 10 and 30 mg, and also placebo as a control, were given each as an intravenous bolus to normal volunteer human subjects; as observed in our previous studies, an apparent "ceiling" effect was observed. The extent of activation of the hypothalamic-pituitary-adrenal axis was not significantly greater with 30 mg of naloxone administered intravenously, as contrasted with 10 mg dose (82). In these studies which are continuing, nalmefene appears to have both a more pronounced effect on hypothalamic-pituitary-adrenal axis activation, suggesting kappa receptor, as well as mu receptor activation may be involved; also, a prolonged effect of nalmefene was observed in human volunteers, which was expected due to the different longer-acting pharmacokinetic profile of nalmefene as compared with naloxone in humans

(77,78,82,83). These findings may have considerable implications for human neurobiology and neuroendocrinology, as well as stress responsivity.

Other studies conducted in patients with specific addictive diseases in our clinical research, both in patients undergoing opiate withdrawal and former opiate addicts managed with a specific opioid antagonist such as naltrexone, have extended some of the earlier observations about the role of stress responsivity during opiate withdrawal. Many groups had observed an apparent activation of the hypothalamic-pituitary-adrenal axis, as evidenced by increased amounts of plasma or urinary glucocorticoids, in heroin addicts under going spontaneous opiate withdrawal (or detoxification using low doses of various medications which do not completely prevent withdrawal symptoms); these findings suggest that the stress of opiate withdrawal causes activation of the hypothalamic-pituitary-adrenal axis (45,58).

However, findings from several studies from our laboratory and in our collaborations suggest that activation of the hypothalamic-pituitary-adrenal axis may precede or occur simultaneously with some of the subjective measures of opiate withdrawal (46,64,84,85). Studies performed in collaboration with Kosten and Rosen at Yale, in the setting of precipitation of very modest opiate withdrawal symptoms by administration of a small dose of opioid antagonist during the late detoxification period, (research performed as part of an effort to develop more diverse medications for management of opiate withdrawal in different settings), it was found that activation of the hypothalamic-pituitary-adrenal axis occurred at the same time as the patients first reported symptoms of opiate withdrawal and at the time of the first appearance of cardiovascular changes of increased heart rate but, provocatively, before any clinical observer documented onset of signs and symptoms of opiate withdrawal and prior to the most severe of the patient reported symptoms (84,85).

These findings extended the much earlier findings from our laboratory which were conducted in a very different research paradigm (64). In the course of metyrapone testing in stabilized methadone maintained patients, it was found that signs and symptoms of opiate withdrawal occurred in many subjects within 30 minutes to one hour after metyrpone administration; these symptoms lasted for a short period of time of one to two hours, much less than that of the pharmacokinetic profile of metyrpone itself (64). Plasma levels of methadone were found not to change due to metyrapone administration. It was hypothesized that one possible explanation for this onset of classical opiate withdrawal symptoms for a limited period might be due to the fact that an internal cue of opiate withdrawal was perceived and that this cue was the activation of the hypothalamic-pituitary-adrenal axis, probably entailing both enhanced levels of corticotrophin releasing factor (CRF), as well as the measured increases in peripheral levels of ACTH and beta endorphin, all of which accompany the abrupt blockade of cortical synthesis. The elevation of these stress responsive hormones might act as an internal cue to contribute to the onset of withdrawal symptoms, since these dramatic changes are a major correlates of both spontaneous and naloxone-precipitated opiate withdrawal in opiate dependent patients, and since most of these stabilized methadone maintained patients would have experienced many episodes of opiate withdrawal while heroin addicts, prior to entering methadone maintenance treatment (64).

We have also observed activation of the stress responsive hypothalamic-pituitary-adrenal axis prior to the onset of patient reported or clinical observer recorded opiate withdrawal symptoms in a study of chronic pain patients with constipation, in whom increasing oral doses of minimally systemically bioavailable naloxone were administered in a research titration study, to optimize the therapeutic effects of this antagonist on gastrointestinal motility (46).

In other studies conducted in long-term heroin addicts who, following detoxification from heroin, were treated with a specific opiate antagonist, naltrexone, studies conducted in collaboration with the earlier Yale group of Kleber and Kosten, we found that even during modest to moderate term chronic naltrexone treatment (mean time in treatment 5 months), activation of the hypothalamic-pituitary-adrenal axis was found to occur after naltrexone administration, with no habituation or tolerance to this effect (86–88). Specifically, morning plasma levels of beta endorphin were elevated as compared with normal healthy control subjects and both morning and afternoon levels of cortisol were also elevated in naltrexone treated patients (86,87). A small subset of subjects, in whom we were able to study hypothalamic-pituitary-adrenal axis function both during treatment and then after cessation of naltrexone treatment, thus allowing use of each subject as his or her own control, we found that there was persistent activation of the hypothalamic-pituitary-adrenal axis during chronic naltrexone treatment which disappeared when naltrexone medication had been discontinued, and a medication-free interval had elapsed, and when these former naltrexone patients were in a basal state (86,87). However, it should be noted that these same types of subjects, that is, medication-free, illicit opiate-free former heroin addicts, have been shown by our laboratory

to usually exhibit a hyper-responsivity to stressors when challenged with the metyrapone test (86,87).

We have continued more recently to compare the effects of buprenorphine, naloxone and methadone in a group of former heroin addicts in early treatment (88). As has been found by many other studies, and hypothalamic-pituitary-adrenal axis function is abnormal during cycles of heroin addiction; normalization occurs during methadone maintenance treatment. During buprenorphine treatment, if it were preceded by methadone treatment, normal hypothalamic-pituitary-adrenal axis function was preserved; but if buprenorphine was the primary treatment, during the very short period of management with buprenorphine, complete normalization of the hypothalamic-pituitary-adrenal axis was not observed (88).

In another line of studies we have had the opportunity to study the impact of chronic stress of an unavoidable type on hypothalamic-pituitary-adrenal axis in humans, as has been studied in work by other laboratories, particularly by Akil and Young of the University of Michigan. We have found a lower than normal level of hormones of the hypothalamic-pituitary-adrenal axis, including plasma levels of beta endorphin and cortisol, in chronic spinal cord injured individuals (89–90). In further studies in this human model of unremitting stress, it was found that during functional therapeutic electrical stimulation, neuroendocrine changes were observed, with increasing partial normalization of this hypothalamic-pituitary-adrenal axis function (90,91).

In very recent studies we have extended our earlier clinical research observations of hyper-responsivity to metyrapone-induced stress and confirmed that such hyper-responsivity may exist in recently abstinent cocaine addicts; further studies are continuing to further elucidate this phenomenon and its mechanism. Also, laboratory studies continue to be conducted to further explore each of these stress responsivity addictive disease related phenomena.

Over the past several years, several hypothalamic-pituitary-adrenal axis peptides and receptors have been defined and the genes cloned, for instance, by the group of Vale at the Salk Institute, for the receptor and gene for CRF, the major regulator of proopiomelanocortin (POMC) release in humans and other mammals, and more recently, for CRF receptors of at least two different types. Using riboprobes constructed from cDNAs from these genes and a modified technique of solution hybridization RNase protection assay, we have recently been able to extend work by quantitative studies performed by many other laboratories studying the normal regulation of the hypothalamic-pituitary-adrenal axis. In our studies conducted by Zhou and colleagues, we have measured both the actual levels expressed in pg/μg (pg mRNA of gene of interest in μg total RNA in region) in specific brain regions, where there was localization of CRF and CRF-R1 type receptors and also studied the differential regulation in specific brain and pituitary regions of these genes, as well as of POMC genes by peptide and steroid hormones of this axis (92). In these studies it was reconfirmed that a glucocorticoid, dexamethasone, will effect decreased gene expression (decreased mRNA levels) in the hypothalamus of CRF and also decreased mRNA levels for POMC in the anterior and intermediate lobes of the pituitary.

However, neither CRF gene expression or POMC gene expression was altered in other brain regions where they were found, including no regulation by dexamethasone of POMC mRNA in the hypothalamus or in the amygdala (where measurable levels of POMC mRNA were found) and no regulation by dexamethasone of CRF mRNA levels in the amygdala, olfactory bulb or frontal cortex (92). It was also found that the glucocorticoid dexamethasone "down-regulated" or caused a decrease in levels of mRNA for the CRF-R1 receptor gene in the anterior pituitary but had no effect on CRF-R1 in mRNA levels in the intermediate lobe of the pituitary or in the amygdala, olfactory bulb or frontal cortex (92). Also, these studies confirmed many earlier studies, that CRF administration results in increased levels of mRNA of POMC in the anterior pituitary, but not in other regions (92).

In further studies, we addressed the question of whether or not methadone delivered by pump to achieve the long-acting, sustained properties which it has in humans, would alter expression on mRNA levels of genes of this stress responsive axis. In these studies the amounts of methadone used were adequate to achieve plasma levels in the rats similar to those seen in low to moderate dose methadone maintenance treatment in humans. When methadone was infused in this manner for seven days, no alterations in levels of gene expression of CRF, CRF-R1 or POMC were observed (71).

Thus, in the rodent model these studies have extended earlier studies documenting that glucocorticoids act in a negative feedback mode both in the hypothalamus to regulate CRF gene expression, as well as in the anterior pituitary to regulate POMC gene expression, and also, a new finding was made that glucocorticoid may act at the anterior pituitary to regulate CRF-R1, a finding which has recently also been made by other laboratories. The studies using methadone delivered by pump infusion showed that steady state enhanced perfusion of mu opioid receptors with an opioid has no

effect on expression of these genes of the stress-responsive neuroendocrine axis. However, enhanced release of peptide and steroids of the hypothalamus-pituitary-adrenal axis have been shown to occur both in humans, as well as in animal models, when activated by specific opioid antagonists acting primarily at the mu, but also possibly at the kappa opioid receptors.

All of these findings provide evidence that the stress responsive axis in humans, and also, in animal models, in part, is under negative feedback modulation or control both by glucocorticoids, and also by the endogenous opioids, directed at mu and kappa opioid receptor systems. These findings suggest that following administration of an exogenous opioid acutely, or following intermittent administration of a short-acting opioid on a chronic basis in humans, such as occurs in cycles of heroin addiction, may further attenuate this stress responsive axis by the same negative feedback mechanism levels, accompanied by attenuated circadian rhythms of levels of hormones released by the hypothalamic-pituitary-adrenal axis. The relationship of the endogenous opioid receptor system and the other components of the classical hypothalamic-pituitary-adrenal stress responsive axis in the acquisition and persistence of addictive diseases and relapse to addictions continues to be studied in our laboratory and in many others.

We have extended the molecular biological studies to determine the effects of "binge" pattern cocaine administration on mRNA levels of genes of the hypothalamic-pituitary-adrenal stress responsive axis. Our laboratory and others have suggested individual differences in responsiveness to stress and stressors may contribute to the acquisition of addiction, but at the same time, that cocaine itself may activate the stress responsive hypothalamic-pituitary-adrenal axis (64–69,72). In recent studies utilizing acute administration of cocaine in a "binge" pattern, with three doses administered one hour apart, to model the human pattern of cocaine self-administration, and administered before the predominant sleep period in the rat, to parallel the human pattern of primarily evening "binge" administration of cocaine, we found (as others have using very different experimental paradigms), that cocaine administration causes a highly significant increase in serum corticosterone levels (70,93). Also, we have found that this pattern of cocaine administration for one day results in a very significant increase in mRNA levels for the CRF gene in the hypothalamus, and also significantly increased CRF mRNA levels in amygdala and olfactory bulb (70,93). After two days and three days of cocaine "binge" administration, elevations in serum corticosterone levels continue and these levels in fact become higher than after the first day of cocaine administration (93).

However, after 14 days of "binge" pattern cocaine administration, we found that corticosterone levels though elevated above control levels were significantly lower than after the first few days of "binge" pattern cocaine administration (93). Also, and of great interest, CRF mRNA levels following 14 day "binge" pattern cocaine administration were significantly lower than baseline levels, and much lower than those observed initial earlier cocaine administration. It is possible that this chronic 14-day "binge" pattern cocaine administration is beginning to mimic what has been observed by several groups, including our own, in the setting of chronic, unremitting stress of a variety of types in both animal models and in humans, and also in the post-traumatic stress disorder, that is, a depressed state of function of the hypothalamic-pituitary-adrenal axis, rather than activation of this axis; and also significantly increased CRF mRNA levels in the amygdala and olfactory bulb (as determined primarily by elevation of lowered levels of serum cortisol in humans or corticosterone in the rodent models). All of these findings suggest that an atypical responsivity to stress may pertain, with different observations made at different stages of cocaine, or other stressor, exposure.

Possible Involvement of Opioid Receptors in Three Addictive Diseases: Heroin Addiction, Cocaine Addiction and Alcoholism

Studies from my laboratory conducted to determine the possible role of the endogenous opioid system in opiate addiction, primarily findings from human studies and, more recently, studies in a rodent model, have documented that opiate addiction may disrupt many aspects of physiology normally under control of, or modulated by, the endogenous opioid system. Many of these short-acting opiate-induced abnormalities include disruption of gastroenterological function and immune function, all of which may involve the effects of exogenous opioids as used in cycles of addiction superimposed on the endogenous opioid system. These abnormalities are of great importance for normal physiology, and may contribute to several disease entities, both of infectious disease type, for instance, hepatitis B, C, delta, G and AIDS, as well as gastrointestinal dysfunction, but probably are not related directly to the neurobiology of addiction. However, it is the disruption of the endogenous opioid system, by opiates interacting with the important neuroendocrine systems and impacting upon the normal role of the endogenous opioid system, specifically in the

hypothalamic-pituitary-adrenal stress responsive axis, and also, in hypothalamic-pituitary-gonadal reproductive biology axis, that may contribute to the actual acquisition of drug-seeking behavior and addiction, the persistence of addiction and the relapse to use of addictive drugs following restoration of the drug-free state. There are many other indications from pre-clinical laboratory studies from many research groups suggesting a possible role of the endogenous opioid system in the problem of opiate addiction, including many studies on disruption on the signal transduction systems that communicate the activation of specific opioid receptors. Such disruptions may, in turn, alter many other aspects of neurotransmitter-neuropeptide function, as well as the actual molecular biological control of expression of diverse genes which may be involved in the addictive disease process.

With the successful cloning of the opioid receptors and the availability of cDNA probes which may then be subcloned to produce riboprobes of various sizes and selective sites for hybridization, many other studies have been able to be performed by my laboratory and numerous others. We have asked specific questions about the role of the endogenous opioids in opiate addiction and also during treatment with the synthetic opioid methadone, with its long-acting pharmacokinetic profile in humans. Many earlier studies by numerous investigators have repeatedly found that chronic administration of a specific opioid antagonist such as naltrexone causes significant increase or "upregulation" of opioid receptor binding density as measured either by classical binding studies in vitro or by techniques of quantitative autoradiography with selective opioid ligands. Whether or not this "upregulation" or increase in density of opioid receptors occurs during chronic treatment in humans with the specific opioid antagonist such as naltrexone in the adjunctive treatment of addiction is not yet known, nor is it known whether there is any clinically significant enhanced sensitivity to opioids in a chronic opiate antagonist treated person. However (as discussed above), we have shown that there is both acute and chronic disruption of the important hypothalamic-pituitary-adrenal stress responsive function during opioid antagonist administration (86–88, 94).

We have addressed the question of whether or not chronic opioid antagonist administration, using the mu-preferring opioid antagonist naltrexone which also has some binding at both delta and kappa opioid receptors, will alter the mRNA levels for the mu opioid receptor gene. In these studies, naltrexone was administered on a chronic basis by pump infusion in a rat model; parallel studies were also conducted at the intramural program of NIDA, and the results of the two studies have been reported together (95). Using both the modified technique of solution hybridization RNase protection and other techniques for assessing mRNA levels for the mu opioid receptor, no alteration in mRNA levels for the mu opioid receptor were found during chronic naltrexone administration (95).

In other ongoing studies, we are determining the effects of chronic exposure to the opioid methadone on mRNA levels for the mu opioid receptor gene. Earlier studies from our laboratory have shown that methadone has a very short half-life in rodents, with approximately a 90 minute half-life in rats and a 60 minute half-life in mice (96, 97). Therefore, Unterwald and colleagues delivered methadone by a steady state pump infusion in a rat over a 7-day period. Preliminary, findings from this ongoing work has shown that methadone does not significantly alter mRNA levels for the mu opioid receptors when delivered in a steady state in rodents, to mimic the long-acting properties which pertain in humans, in whom the half-life of methadone is over 24 hours for the racemic mixture used in treatment, and 48 hours for the active enantiomer (98).

In other studies, currently ongoing in collaboration with the several groups of the NIH, including the PET Imaging Group of Dr. W. Eckelman, and with Dr. K. Rice, we have shown no unanticipated alterations in mu opioid receptor binding in humans who are former heroin addicts in long-term methadone maintenance treatment, as determined by positron emission tomography (PET) imaging using $^{[18]}$F cyclofoxy as the radioactive ligand, only the anticipated differences due to methadone occupancy (99). Therefore, although alterations in opioid receptor gene expression and/or presentation of receptor peptides, or the endogenous opioid peptides, and the multiple related single transduction systems, may be altered during cycles of exposure of intermittent high levels of short-acting exogenous opiates, such as heroin, followed by opiate withdrawal in tolerant and dependent individuals, these studies all further support the multiple clinical research findings suggesting that stabilization and normalization of the endogenous opioid system and related aspects of physiology occur during steady exposure to the long-acting opioid methadone. Very recently, we have started to study opioid receptor gene expression levels in human brains obtained in post mortem examinations, using riboprobes subcloned from cDNA from cloned human opioid receptors (100).

Over the past several years, many important clinical research studies had been reported which build upon the earlier numerous, and often conflicting reports of the

role of the endogenous opioid system in alcohol self-administration models, primarily in rodents. Despite many conflicting reports as to the effects of specific opioid antagonists such as naltrexone on alcohol drinking behavior in various animal models, in carefully constructed, placebo-controlled, double-blinded studies, the group of Volpicelli and O'Brien at University of Pennsylvania has convincingly documented that around 50% of chronic alcoholics may be managed successfully, at least for a limited time of up to three months, with regular administration of the specific opioid antagonist naltrexone (101,102). These studies have been further corroborated by studies from the groups of O'Malley at Yale University and also Mason at the University of Miami, using the opioid antagonists naltrexone and nalmefene, respectively (103,104). Many other studies have been performed and are currently in progress to determine the specific roles of the endogenous opioid system in alcoholism, and also, the mechanisms by which specific treatment with specific opioid antagonists may be effective in many alcoholic subjects (61,105,106).

Further clinical research and related laboratory studies in which opioid antagonists and also other provocative tests such as metyrapone, are used to probe the integrity of the stress responsive hypothalamic-pituitary-adrenal axis by disrupting mu, and possibly also kappa, opioid receptor function in both drug- and medication-free former heroin addicts, former cocaine addicts, as well as in former alcoholics, as contrasted to normal volunteer subjects will continue to provide information about the possible role of the endogenous opioid system in these disorders, including several from our laboratory (88, 94,107–110).

Over the past several years our laboratory has also focused on the question of the possible role of the endogenous opioid system in cocaine addiction in several related studies conducted using our ''binge'' pattern model of cocaine administration (93,111–123).

Cocaine has been known for some time to act primarily by binding to the dopamine transporter, thus, inhibiting the pre-synaptic re-uptake of dopamine and yielding an increase in synaptic (and extracellular fluid, in general), levels of dopamine. These surges in synaptic levels of dopamine have also been related to the reinforcing or rewarding effects of many drugs of abuse, and in particular cocaine and other stimulants. A primary site of action of cocaine has been defined to be in the nucleus accumbens, which is the apparent center of the rewarding properties of cocaine. Other sites of cocaine action include the entire mesolimbic-mesocorticol dopaminergic system, where there are dopaminergic terminals, as well as in nigrostriatal dopaminergic system and possibly also in the hypothalamic-tuberoinfundibular-dopaminergic system. In addition, cocaine also has action at the specific serotonin reuptake, transporter epinephrine and the reuptake transporter proteins. The precise role of each of these neurotransmitters in the effects of cocaine continue to be elucidated. Similarly, the role of various, specific post-synaptic, as well as pre-synaptic dopaminergic receptors in cocaine dependency have been, and continue to be studied by many laboratories using many different experimental approaches.

Many of the changes on dopaminergic function, though initially dramatic, subside within a few hours after each administration of cocaine, as has been documented in our ''binge'' pattern cocaine administration model. In the mid-late 1980's, we developed a ''binge'' pattern administration model in rats to mimic the most common human pattern of cocaine abuse, with several doses of cocaine self-administered over a limited temporal period, followed by a long period of no cocaine administration. We have used this model for extensive studies of a neurochemical, molecular biological, cellular biological and behavior types, with multiple investigators using the same animals or parallel animal models to ask specific questions about the effects of cocaine under a peptide neurotransmitter functions, as well as related behaviors (111–123).

Unterwald and colleagues in my laboratory found that this pattern of chronic cocaine administration significantly alters the density of mu opioid receptors in brain, specifically with increased density of mu opioid receptors in those brain regions where there are abundant dopaminergic terminals, including the nucleus accumbens, the anterior cingulate, and the basolateral amygdala of the mesolimbic-mesocorticol dopaminergic system, as well as in the caudate putamen of the nigrostriatal dopaminergic system (112). Very excitingly, a scientific group from Johns Hopkins and the intramural program of NIDA have recently reported similar findings of enhanced mu opioid receptors using PET technology and ^{11}C-carfentanyl binding and the technique of positron emission tomography (PET) (124). In further studies, Unterwald and colleagues also found that chronic cocaine administration caused an increase in density, as determined by the technique of quantitative autoradiography of kappa opioid receptors, again in selected brain regions with abundant dopaminergic terminals including the caudate putamen, the nucleus accumbens, the anterior cingulate, and also the olfactory tubricle (119).

In related studies, we have used the molecular biological technique of modified solution hybridization RNase protection assay to determine the acute and

chronic effects of "binge" pattern cocaine administration on levels of expression of genes of the endogenous opioid system. Using the technique of modified solution hybridization RNase protection assay, Branch and colleagues found that chronic 14-day "binge" pattern cocaine administration did not alter preproenkephalin mRNA levels in specific brain regions of rats (111). However, in subsequent studies, using the "binge" pattern cocaine administration we have found that there are early transient changes in proenkephalin mRNA levels and that these may be blocked by administration of specific D_1 and D_2 receptor antagonists (123,125). In related studies, Spangler and colleagues have found that chronic "binge" pattern cocaine administration induces an increase of prodynorphin mRNA levels, specifically in the rat caudate putamen (113,116). Similar findings have been made using a cocaine self-administration model by Hurd and colleagues at the NIH and in Sweden, and McGinty and colleagues in North Carolina and, more recently, using a similar "binge" pattern cocaine administration model by McGinty and colleagues (126–129).

Also, of considerable interest with respect to the role of the dynorphin-kappa opioid receptor system in cocaine dependency, Spangler and colleagues have recently reported that chronic "binge" pattern cocaine administration results in a significant reduction in levels of mRNA for the kappa opioid receptor and very specifically in the substantia nigra, a region where one would anticipate the greatest impact of dynorphin peptides resulting from enhanced dynorphin gene expression in the caudate putamen due to the dynorphinergic pathway which projects from the caudate putamen to the substantia nigra (122). In further studies, we have found that the effect of "binge" pattern cocaine administration on dynorphin mRNA levels occurs acutely after a single day of "binge" pattern cocaine administration and persists during subacute and chronic "binge" pattern cocaine administration. However, no significant changes in kappa opioid receptor mRNA levels after acute cocaine administration (125).

Extending those studies, into a different animal model, LaForge has recently subcloned the enkephalin gene from guinea pigs and is currently using this cloned gene to determine the effects of cocaine on enkephalin gene expression in that species (130). Also, Yuferov has recently cloned the guinea pig preprodynorphin gene and is currently using resultant subcloned riboprobes in studies of the cocaine effects on prodynorphin gene expression in the guinea pig (131,132).

Many other neuroanatomical, neurobiological studies have documented the probable linkage between D_2-type dopaminergic receptors with enkephalinergic neurons, and of more importance for many of our findings, also the linkage of D_1-type dopamine receptors with dynorphinergic neurons. All of these findings are particularly provocative coupled with the early findings of Sivam at Indiana University School of Medicine, that cocaine selectively increases striatonigral dynorphin levels (133).

Further molecular cell-biological, neurochemical and behavioral studies will be conducted in various animal models, as well as humans to determine the possible role of the endogenous opioid system in general, and in particular, the dynorphinergic-kappa opioid receptor system in cocaine dependency. Also, we will continue to study the possible utility of dynorphin A peptide-like kappa agonists for the modulation of dopamine basal levels and surges and for the possible management of cocaine dependency in humans (117,134–139).

CONCLUDING COMMENTS

The career commitment of Dr. Eric Simon and his colleagues and his many successful trainees in areas focused on defining the opioid receptors and determining their various activities, including their possible relevance to narcotic addiction, have exemplified the pathway of an exciting scientific career, one of focus, and yet, one of unearthing the unexpected and pursuing with great vigor the explanations and mechanisms underlying both the predicted and more serendipitous findings. It is clear that the career of Dr. Eric Simon underscores the great value which can come from interactions between scientists, both of the actual collaborative research type, but also those which entail a close intellectual communications and working together for a common cause of great need. The early volunteer activities, which undoubtedly were both time consuming and at times, undoubtedly frustrating, of Dr. Eric Simon for the New York City Health Research Council Working Group on Narcotic Addiction, from 1960 to 1964, benefited the city, the state and the nation. These important volunteer activities also enriched and enlarged the vistas of all those working or interacting with Dr. Simon and his group, then and to this present day. His courage, his kindness, his integrity, and his great willingness to share, have made Dr. Simon a wonderful colleague, highly respected and enjoyed by us all who have had the opportunity to interact with him.

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