

NATHAN B. EDDY MEMORIAL AWARD LECTURE

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MEDICINAL CHEMISTRY IN THE STUDY OF ADDICTIVE DISEASES

First let me say that it is extremely gratifying to stand here as the 28th recipient of the Nathan B. Eddy Award and also as chief of the research group established by Dr. Eddy more than 60 years ago at NIH. At the same time, I am almost overwhelmed with humility and gratitude to those who helped me achieve our goals during the last 27 years. This is without a doubt the high point of my career and I am extremely grateful to have been selected.

I would like to thank my nominator, Dr. Jim Woods, and also Drs. John Lewis and Everette May for supporting my nomination, the Awards Committee that selected me as recipient and all of CPDD. I am indebted to my long-term NIH colleagues Drs. Arthur Jacobson and Richard Rothman and Ms. Mariena Mattson. I would also like to thank Nancy Lew for her long-term support. Much of the chemical work I will describe was done by about 60 highly talented postdoctoral fellows from 16 countries. It has been a privilege to work with numerous others that have made many important contributions in the biological study of compounds synthesized in our group. Time does not permit me to mention each person and their contributions but to those of you in the audience and those of you that are not, let me say thank you very much. I am greatly appreciative of the long-term support of my program by the National Institute of Diabetes, Digestive and Kidney Diseases and for additional support provided by the National Institute on Drug Abuse. I am indebted to Dr. Carl White and Mallinckrodt, Inc. for long-term and generous contributions of starting materials required for our studies. I am also thankful to have been a student of my Ph.D. advisor, the late Dr. John Dyer. Dr. Dyer, together with Drs. Daniel Dickel and the late Harry Petree of Ciba-Geigy Pharmaceutical Co. (now Novartis), largely trained me in organic chemistry. The training I received under Dr. Dickel's tutelage in the practice of organic chemical synthesis during 1971-1972 has served me well during my career and substantially contributed to the development of the NIH Opiate Total Synthesis.

I am very grateful to two great scientists of drug abuse research, Drs. Nathan Eddy and Dr. Everette May. First to Dr. Eddy who more than any other person was responsible for the foundation on which I began my program. Second, to my mentor in this field, Dr. May, for his confidence in giving me a chance to work in his group and for his guidance, sound advice and friendship for the last 27 years. Without his help, I would have had no chance to be standing here today. I attended my first CPDD Meeting and also the International Narcotics Research Conference in 1975 as a postdoctoral fellow in Dr. May's group at the NIH. At these meetings, I was in awe of Dr. May and the other leaders of the field including Dr. Harris Isbell, the Eddy Award recipient that year. Having been trained only in organic chemistry, this was the first time I began to fully appreciate the power of the combination of organic chemistry and pharmacology.

It never crossed my mind that I might someday become the Eddy Award recipient. What did cross my mind was that organic chemistry could play a powerful part in drug abuse research and that I would try my best. In retrospect, I was very fortunate to have entered the field just one year after biochemical identification and binding assays for the "opiate receptor" (then singular) were published and at the time when the endogenous ligands were first identified. I was in attendance at Arlie House in 1975 when Dr. Hans Kosterlitz described two peptides that had been isolated from brain and were active in opioid receptor assays. The structures of these peptides were subsequently elucidated as methionine and leucine enkephalin and the findings published in *Nature* in December 1975. This work, together with the biochemical identification of the receptor, provided much insight into the mechanism of action of opioids at the time. It also greatly extended the opportunity for investigation from what were largely whole animal and isolated tissue studies with some human studies to the quantitative *in vitro* investigation of the receptors and their function. What were needed at that time were diverse chemical tools for elucidation of the structure and function of the opioid receptor-endorphin system. I was certain that with my prior training in organic chemical synthesis I could make a contribution to the field.

Now, as I stand before you almost 20 years to the day after Dr. May received the 1981 Eddy Award, I will relate some highlights of our program which were greatly influenced by my two years as a postdoctoral fellow with Dr. May. First, I would like to briefly mention 11 research areas that we have been involved with since I began in 1974. I will then discuss, in more detail, our work in four of these areas, (a) unnatural opiates and the opiate total synthesis (b) development of cyclofoxy as a PET ligand (c) delta opioid receptor selective ligands and (d) stimulant abuse-treatment and prevention.

Our contributions to the chemistry of the opium alkaloids were generally aimed at the advancement of our synthetic goals whether this involved improvement of existing synthetic routes by necessity or the development of novel methodology. We developed an improved N-demethylation of morphine and codeine (Rice 1975, Rice and May 1977) and I described a high-yield, boron tribromide mediated O-demethylation of codeine to morphine (Rice 1977), with both methodologies applicable to most other opiate and opiate-related compounds. High-yielding opiate O-demethylation of codeine to morphine was unavailable at the time and was required for our syntheses of unnatural opiate enantiomers from the small amounts of sinomenine then available (see below). This remains an important general transformation in the synthesis of experimental and clinically useful drugs today. Although the O-demethylation was widely applicable, it did not work on compounds highly sensitive to acid conditions such as the thevinols and thebaine itself. We later introduced L-selectride as a convenient reagent for basic O-demethylation of thebaine to oripavine, a transformation that had been unsuccessfully attempted for 60 years (Coop *et al.* 1996, Coop *et al.* 1998). This reagent also proved successful for the acid-sensitive thevinols (Coop *et al.* 1998). This technology is highly complementary to the boron tribromide O-demethylation of opiates I developed earlier and this combination can thus serve for most O-demethylations of opiate and opiate-related compounds. We also developed a practical, direct oxidation of codeinone to 14-hydroxycodeinone, a novel synthesis of thebaine from codeine and a facile synthesis of thebainone-A and dihydrothebainone from codeine. Finally, with regard to our contributions to the chemistry of the opium alkaloids, we developed the NIH Opiate Total Synthesis that will be discussed in more detail below. This is the only practical methodology for the total synthesis of opium-derived medical narcotics and

their antagonists and is now in manufacturing process development. Oxide bridge closure in the N-nor series, high-yield oxidation of northebaine to 14-hydroxynorcodeinone and other transformations were developed in this work that greatly extend the versatility of the total synthesis.

Much of our work has involved the design and synthesis of novel drugs as research tools, imaging agents and potential drugs for numerous biological targets. A recurring theme throughout my work in drug abuse research has been the effect of stereochemistry on drug activity. One of my earliest projects in this area was a 1976 study of the antinociceptive effects of thujone and its derivatives (Rice and Wilson 1976). Thujone is a major active constituent of absinthe, an intoxicating essential oil preparation popular in Europe in the period around 1900 that produced very bizarre effects in certain individuals. This project originated after a paper appeared suggesting that the effects of absinthe resulted from the action of thujone enol on the same receptor (then unknown) as Δ^9 -tetrahydrocannabinol (Δ^9 -THC), the major active principal in marijuana. I recognized that this could not be the case since the absolute configurations of isothujone and Δ^9 -THC were known and this hypothesis would require the unnatural enantiomer of thujone enol. Since a commercial mixture of (-)-3-isothujone and (+)-3-thujone did however, show significant antinociceptive activity like Δ^9 -THC, we prepared pure samples of (-)-3-isothujone, its racemate, its epimer (+)-3-thujone and a number of related compounds and studied these for antinociceptive activity in the same systems that Δ^9 -THC is active. Our results showed that (-)-3-isothujone was the most active compound studied being about twice as active as the racemate and substantially more active than the other compounds in the series and that it produced convulsions at higher doses. Based on these observations of structural and stereoselectivity in the antinociceptive activity of (-)-3-isothujone, we suggested that a specific receptor interaction could be involved. A recent rigorous study published by others showed that (-)-3-isothujone acts as a functional antagonist of the GABA_A receptor complex and produces convulsions similar to picrotoxin (Hold *et al.* 2000).

We designed and synthesized a number of tools for the central benzodiazepine receptor complex including the first electrophilic affinity label (Rice *et al.* 1979), β -carboline as inverse agonists, chiral barbiturate enantiomers, chloride channel affinity labels and fluorescent ligands. We studied peripheral benzodiazepine receptor ligands and prepared affinity label enantiomers and an iodinated ligand as a potential imaging agent for single photon emission computed tomography (SPECT) of the peripheral receptor. Our work in the "diazepam insensitive receptor" system involved the design and synthesis of a [¹¹C]-labeled drug as a potential agent for positron emission tomography (PET), a [¹²³I]-labeled ligand as a potential SPECT agent, and other analogs.

Our studies also involved the design and synthesis of ligands for study of the mechanism of action of phencyclidine (PCP), a drug that produced unpredictable, sometimes violently aggressive behavior in certain individuals. We designed and synthesized enantiomeric pairs of PCP derivatives and related compounds, the affinity labels metaphit and fourphit, [¹¹C]MK-801 and [¹⁸F]TCP as potential PET imaging agents and numerous other analogs for various studies. We showed that metaphit antagonized the actions of PCP in single cerebellar cell preparations (Wang *et al.* 1986).

In the cannabinoid-receptor area, we initiated a study to map the anatomical distribution of cannabinoid receptors using tritiated CP55,940 a non-classical, high-affinity cannabinoid ligand originated at Pfizer by Drs. Ross Johnson and Larry Melvin (Herkenham *et al.* 1990). These studies provided insight into the question of why large doses of Δ^9 -THC are non-toxic and a foundation for further work that continues today. We also designed and synthesized affinity labels for the cannabinoid receptor based on the aminoalkylindoles developed at Sterling and CP55,244, another non-classical cannabinoid developed in the Pfizer program. We recently reported a novel class of CB1 and CB2 cannabinoid receptor ligands.

Our studies have resulted in many novel compounds in a variety of structural classes that act on sigma recognition sites. Among these, we described the first radiochemical synthesis of [3 H](+)-pentazocine, an important radioligand for the sigma-1 recognition site and sigma-2 selective ligands from the indolophenylmorphans class.

Most recently, we have studied corticotropin releasing hormone (CRH) receptor antagonists. This system mediates adaptation to stress, and plays a role in the pathogenesis of anxiety and major depression and is involved in drug dependence and withdrawal. Our goals in this area included the development of functional PET and SPECT imaging agents for the CRH receptor and the design and synthesis of other ligands for various studies related to this system. Among these, we synthesized more than two kilograms of a compound originated at Pfizer, that we named antalarmin. This work provided material for many studies that have provided substantial insight into the CRH receptor system. We showed that antalarmin attenuated the CRH mediated stress responses in primates in the intruder paradigm, an intense social stressor (Habib *et al.* 2000). Our results indicated that antalarmin blocks the ability of CRH to promote blastocyst implantation and early maternal tolerance of pregnancy (Makrigiannakis *et al.* 2001). We synthesized a high affinity fluorinated ligand for the CRH receptor and tritiated this compound. Other studies provided a subnanomolar affinity fluorinated analog as a potential PET ligand and iodinated ligands as potential SPECT imaging agents for the CRH receptor. As part of this program, we developed a practical synthesis of precursors of [11 C]MDL100,907, a 5-HT_{2A} receptor antagonist required for our CRH receptor studies.

One of the major research areas we have been involved with since 1974, is the development of novel research tools for study of the opioid receptor system. When I began in Dr. May's group, my first assignment was to synthesize the racemic and chiral 2,5-dimethyl-2'-hydroxy-9 α - and β -propyl-6,7-benzomorphans and other derivatives, which were needed for structure-activity studies (Rice *et al.* 1975). As is usually the case, some of the most difficult compounds in a series are generally the last to be synthesized and this was no exception. The problem here was that the required starting material, 3-propyl-4-methylpyridine was not available and several hundred grams were needed. I began my career in opioid research by synthesizing this material from ethyl acetoacetate. Later, we designed and synthesized the selective affinity ligands BIT for the mu receptor, FIT, FAO (Rice *et al.* 1983) and superfit for the delta receptor and UPHIT for the kappa receptor. These compounds proved to be valuable tools for further characterization of the opioid receptor-endorphin system. We tritiated FIT and superfit to high specific activity and employed superfit for purification of the delta receptor to homogeneity from NG108-15 cells (Simonds *et al.* 1985). This enabled the study of the delta receptor eight years prior to the availability of the cloned delta receptor. We also developed iodinated derivatives of naltrexone

as potential SPECT imaging agents. Most of our effort on the development of imaging agents focused on the development of (-)-cyclofoxy [6- β -fluoro-6-desoxynaltrexone]. The [^{18}F]-(-)-cyclofoxy proved successful for human PET imaging studies and will be discussed in greater detail below, as will the role of the NIH Opiate Total Synthesis in the development of cyclofoxy. We also synthesized a number of opioid enantiomeric pairs and tritium labeled some of these, including some of our affinity ligands that were discussed earlier. In other work related to the delta opioid system, we synthesized numerous delta opioid ligands related to SNC80 including [^3H]SNC121 (see below). We identified a novel class of delta selective ligands and a novel structural type of narcotic antagonist based on the 5-phenylmorphans discovered by Dr. May (Awaya *et al.* 1987). In order to gain further insight into the conformational requirements for the opioid activity of some phenylmorphane enantiomers, we introduced the oxide-bridged phenylmorphans as conformational probes (Burke *et al.* 1984).

One other area that we have investigated is the development of drugs for investigation of the mechanism of action of psychomotor stimulants and as potential medications for the treatment and prevention of psychomotor stimulant abuse. We developed methodology for the synthesis of isomeric mono and dinitroimipramines and desmethylimipramines and for tritiation of 2-nitroimipramine. We designed, synthesized and tritiated an azido derivative of GBR12935 as a photoaffinity label for the dopamine transporter protein (DAT) that enabled us to purify the transporter to homogeneity in 1991. We also developed electrophilic and tritiated labels for this site along with numerous other GBR12909 analogs for this site including those nonselective for the biogenic amine sites. Finally, we introduced GBR 12909 as a potential medication for the treatment of psychomotor stimulant abuse and have developed ultra long-acting derivatives of GBR12909 for the same purpose.

UNNATURAL OPIATES AND OPIATE TOTAL SYNTHESIS

One of the first problems that I worked on in Dr. May's group was the synthesis of unnatural (+)-morphine as a tool to study the newly discovered opiate receptor. Dr. Mario Aceto, of the Medical College of Virginia, suggested this problem to Dr. May. Although several opiate total syntheses had been described, the most notable being that of Gates and Tschudi, none were applicable to the synthesis of multigram quantities of unnatural (+)-opiates. It was decided to utilize naturally occurring sinomenine as starting material since this alkaloid had the carbon-nitrogen skeleton enantiomeric with the natural opium alkaloids and had already been converted to (+)-morphine and derivatives by Goto using the methodology of Gates in the late steps. The problem with repeating Goto's work was the limited amount of sinomenine available and the very low chemical yields in the late steps resulting in only about 3% overall yield of (+)-morphine from sinomenine. We planned to develop superior methodology for these steps using the corresponding enantiomers freely available from opium products as model compounds and then to apply these results to sinomenine. Dr. May was able to obtain sinomenine for the program from Tanabe Pharmaceutical Co. who graciously prepared it for us by extraction of *Sinomenium acutum*. We then worked out novel methodology for the conversion of dihydrocodeinone to codeine (Iijima *et al.* 1977) and a rapid high yielding conversion of codeine to morphine (Rice 1977) thus eliminating the low yielding steps of the Goto sequence. We then extended this program to prepare (+)-thebaine and converted it to (+)-naloxone via (+)-oxymorphone. In this work, we prepared multigram amounts of (+)-morphine (Jacquet *et al.*

1977), about a gram of (+)-naloxone (Iijima 1978), and lesser amounts of other (+)-enantiomers. These compounds showed between 10^3 and 10^4 less affinity for the opioid receptors than their enantiomers *in vitro* and in bioassays and showed no opioid effects *in vivo*. These (+)-enantiomers thus proved to be valuable research tools for detecting opioid receptor mediated effects in diverse systems and (+)-naloxone proved essential in our initial PET imaging of the primate opioid receptor as discussed below. Sinomenine later became unavailable and we considered the feasibility of development of a practical total synthesis of opiates. The impetus to develop such a process was two-fold. First, the worldwide opium shortage of 1973-1976 that emphasized the desirability of development of methodology for the production of these drugs by total synthesis as an alternate to reliance on a natural product. This was a severe shortage requiring release of about half of the U.S. strategic materials reserve of opium to domestic processors so that demands for medical narcotics could be met. Four factors contributed to the shortage: (a) Turkey ending production in 1972; (b) Russia buying opium on the world market for the first time; (c) Indian crop failures in 1973-1974; and (d) increased medical demand for codeine. The second consideration was our requirement for a diverse group of unnatural opiates as research tools. Their synthesis would require development of novel methodology and our goal that has now been successively accomplished was defined as follows: A practical total synthesis should: (a) provide 100+ g of optically pure, correctly oxygenated morphinan intermediates per batch beginning on a 1 mole scale in the laboratory; (b) be as short and simple as possible; (c) be clearly amenable to commercial production of any quantity of all opium derived medical narcotics and their antagonists at a reasonable price; and (d) offer independence from natural sources. Regarding the quantity of materials in question, the 2001 Drug Enforcement Administration production quota for all Schedule 2 (medically useful) narcotics for sale was 129,000 kg with about 65,600 kg of thebaine as raw material (for conversion). Since morphine, codeine, and thebaine are the only materials isolated for the production of medical narcotics and their antagonists from opium, a practical synthesis of these materials would allow full access to the entire spectrum of natural and unnatural enantiomers. In 1980, I published a short, non-chromatographic, practical synthesis of racemic nordihydrocodeinone and dihydrocodeinone using a modified Grewe approach. This route employed unprotected phenolic intermediates and novel oxide bridge closure in the N-nor series that provided optional access to either compound in about 30% overall yield from 3-methoxyphenethylamine (Rice 1980). This methodology was then easily adapted to the synthesis of both enantiomers of these compounds thus providing access to chiral morphine, codeine and thebaine using methodology we and others described earlier. Subsequent work in our group has resulted in simplified methodology for the chiral synthesis of either enantiomer of nordihydrocodeinone and dihydrocodeinone. We converted the latter to the following compounds in the indicated yield from 3-methoxyphenethylamine with the number of isolated intermediates (any intermediate filtered and washed) shown in parentheses: dihydrocodeinone 40% (5), codeine 36% (6), morphine 32% (7) and thebaine 35% (5). Since the pharmacologic profile of opiates is largely determined by the substituent on nitrogen, we developed methodology for the nearly quantitative conversion of chiral nordihydrocodeinone to northebaine and for high yield, direct oxidation of the latter to 14-hydroxynorcodeinone (Rice and Newman 1997). Catalytic hydrogenation followed by boron tribromide O-demethylation gave noroxymorphone. This chemistry allows introduction of any desired nitrogen substituent at any stage in the sequence and eliminates the multistep replacement of the N-methyl substituent of morphine, codeine and thebaine with other substituents. It thus greatly extends the versatility of the opiate total synthesis by allowing one intermediate to serve as precursor for various drugs

with different N-substituents. Using this combined methodology, we synthesized the (+)-isomers of oxymorphone, naltrexone, naloxone, nalmefene, nalorphine, etorphine, buprenorphine, diprenorphine, and numerous other unnatural opiate enantiomers. Although we previously confirmed the optical purity of the early tetrahydroisoquinoline intermediate in the total synthesis and thus materials made from it, we independently verified the optical purity of (+)-oxymorphone from the total synthesis by showing its biochemical identity with (+)-oxymorphone from sinomenine in radioreceptor binding assays. We also synthesized unnatural (+)-cyclofoxy, a compound that played an important role in our PET studies. These studies resulted in the first images of opioid receptor occupancy in the living primate brain (see below). In summary, the NIH Opiate Total Synthesis: (a) provides the only practical methodology for the chemical synthesis of all opium derived medical narcotics and antagonists; (b) allows total synthesis of morphine, codeine and thebaine in 32-36% overall yield with only 5-7 isolated intermediates from readily available raw materials; (c) offers an unlimited commercial source of opium-derived narcotics and antagonists independent of foreign sources of opium; (d) enables unlimited production of the unnatural (+)-enantiomers of all opium-derived medical narcotics and antagonists as research tools and drugs; and (e) offers opium poppy eradication as a worldwide strategy for the elimination of illicit heroin production. This technology is now in manufacturing process development and is the only practical methodology available for this purpose despite continuing attempts by many chemists to develop such a process over the last 70 years, and major advances in organic chemical synthesis made during those decades.

THE DEVELOPMENT OF (-)-CYCLOFOXY AS A PET LIGAND

The opioid receptor endorphin system consists of saturable, enantioselective, high affinity mu, delta and kappa opioid receptor types (and at least two subtypes of each) located in anatomically well defined areas of the mammalian CNS and the numerous endogenous opioid peptides (endorphins) which subserves these receptors. This system mediates the euphoric and addictive effects of narcotic drugs and regulates numerous physiologic and behavioral functions in its normal state, whereas its dysfunction likely results in a number of CNS disorders. PET scanning is a unique, noninvasive technique available for real time measurement of metabolic activity or receptor occupancy in living animals and humans and is thus applicable to the study of the function of this system. We designed and synthesized (-)-cyclofoxy as a candidate opioid receptor, PET imaging agent. This compound bound preferentially to mu receptors with some kappa receptor binding with about 1 nM affinity *in vitro*, functioned as a potent narcotic antagonist *in vivo* [about 10 x (-)-naloxone (Narcan)] and, importantly, was not metabolized in the brain. Autoradiographic studies in brain sections of rats using [³H](-)-cyclofoxy revealed that the drug localizes *in vivo* in opioid receptor rich brain regions and labels a population of opioid receptors similar to that labeled by the clinically used narcotic antagonist naloxone. The binding could be removed by washing, was reversible and could be displaced and prevented by (-)-naloxone, but not the pharmacologically inert (+)-naloxone prepared by our total synthesis. The unnatural (+)-isomer of cyclofoxy prepared by total synthesis using methodology described above was also inactive in these systems. In addition, the release of endorphins could be measured with [³H](-)-cyclofoxy in the hamster brain *in vivo* strongly suggesting that [¹⁸F](-)-cyclofoxy might be a useful imaging agent for opioid receptor occupancy in primates. That proved to be the case and we reported the first successful images of opioid receptor occupancy in the living primate brain in 1984 (Pert *et al.* 1984). In this study, we used the 3-acetyl derivative

as a rapidly metabolized prodrug of [^{18}F]-(-)-cyclofoxy. This study in baboons revealed extensive localization of [^{18}F]-(-)-cyclofoxy in the opioid receptor rich caudate nucleus and thalamus analogous to the labeling of these brain regions in the rat with [^3H]-(-)-cyclofoxy. Accumulation of [^{18}F]-(-)-cyclofoxy in these primate brain regions was displaceable by (-)-naloxone (or preventable with prior administration of (-)-naloxone) in a dose-related manner in contrast to the receptor inert (+)-naloxone that had no effect. In our later studies, we used [^{18}F]-(-)-cyclofoxy in order to simplify the tissue distribution analysis. Equilibrium binding studies in conscious humans developed by Dr. Richard Carson at NIH revealed similar localization of [^{18}F]-(-)-cyclofoxy in the caudate nucleus and thalamus analogous to the labeling of these brain regions in the rat and baboon. In order to better understand opioid receptor function and detect more subtle differences between normal and abnormal subjects, we developed methodology for quantitation of opioid receptor density and affinity applicable to human studies. This was achieved by B_{max} and K_d quantitation in 16 primate brain regions through *in vivo* Scatchard analysis of (-)-cyclofoxy binding under rigorously demonstrated equilibrium binding conditions with precise measurement of non-specific binding. The latter was accomplished by two independent methods that were in excellent agreement: (a) PET studies with the receptor-inert mirror image isomer [^{18}F](+)-cyclofoxy above and (b) displacement of specifically bound (-)-cyclofoxy with (-)-naloxone and subsequent measurement of the residual receptor-unrelated [^{18}F]-(-)-cyclofoxy binding, a method easily applicable to human studies. Opioid receptor quantitation in appropriate brain regions of humans in normal, drug-altered and pathological conditions should allow the development of clinical correlates of receptor dysfunction with disease states. These results will enable rapid and routine diagnosis of these disorders, and provide the means to identify and monitor the effects of appropriate drug therapy for disorders of the opioid receptor-endorphin system. The development of (-)-cyclofoxy may have enormous potential for further understanding normal and abnormal brain function including narcotic and cocaine addiction, other drug-seeking behavior, and the “opiate tone” of the CNS which modulates the mesolimbic dopaminergic pathway thought to control the rewarding effects of drug abuse and other behaviors that certain individuals find reinforcing. The first steps toward these goals have been realized with our recent study of normal and methadone maintained former heroin addicts with Dr. Mary Jeanne Kreek and associates (Kling *et al.* 2000). This study provided critical information on the binding of [^{18}F]-(-)-cyclofoxy in 13 brain regions and how this accumulation differs in the methadone maintained subjects.

DELTA OPIOID RECEPTOR SELECTIVE LIGANDS

We began our studies to identify delta selective drugs after the publication of the lead structure of BW373U86 in 1992 by Dr. Robert McNutt then at the Burroughs-Wellcome Laboratories. Pharmacological results for BW373U86, a racemate, indicated that this compound was a high affinity delta ligand that produced some of its effects through the mu receptor. Our initial approach was to prepare the enantiomers of BW373U86 and a series of chiral derivatives attempting to exploit the remarkable effect of stereochemistry on biological activity observed in the opiates. One of the best examples of this is the enantiomers of etorphine. The (-)-enantiomer of etorphine, originally reported by Bentley in 1963, is among the most potent analgesics known, about 7000 times more potent than morphine. By contrast, the (+)-enantiomer (prepared by us via our opiate total synthesis) was inactive in all tests studied for opioid activity and non-toxic at 100 mg/kg, i.p. in the mouse. It is also a potent non-narcotic antitussive about three times the

potency of (-)-codeine. One of the most interesting compounds that resulted from our initial delta ligand synthesis studies was SNC 80, a highly selective delta agonist that shows 2000-fold selectivity in binding and bioassays for delta vs. mu receptors (Calderon *et al.* 1994). This work also provided a number of related drugs that are highly delta selective, for which we established structure activity relationships at the cloned human mu and delta receptors. These studies revealed SNC 162 as one of the most delta selective ligands known with a selectivity ratio of delta vs. mu of 8770. We also synthesized a fluorinated SNC 80 derivative with about 900 fold delta selectivity as a potential ligand for imaging delta opioid receptor occupancy by PET, and [³H]SNC 121 a novel, highly selective delta opioid receptor radioligand with 6000-fold delta selectivity. In other work, we showed that SNC 80 was the most efficacious delta receptor agonist at the cloned human delta opioid receptor among the compounds studied. In studies in the rhesus monkey, we showed that SNC 80 was a systemically active, delta selective agonist with a rapid onset of action and relative low toxicity in comparison to other opioids.

Recent studies by others have shown that moderately selective delta opioid antagonists suppress (a) cocaine seeking behavior, (b) heroin self-administration in rhesus monkeys and (c) the development of tolerance and dependence to the mu agonist morphine. The former two observations strongly indicate that highly selective delta receptor antagonists might be valuable medications for the treatment and prevention of human cocaine and narcotic abuse, and perhaps other undesirable reinforcing behaviors. The latter observation suggests that a drug showing a mu agonist-delta antagonist profile might produce strong analgesia without producing tolerance and dependence, thus allowing continuous treatment of chronic pain without escalating doses and the inevitable side effects that occur. Dr. Peter Schiller has independently presented convincing evidence that compounds with the mu agonist-delta antagonist profile do indeed retain strong antinociceptive effects and show reduced side effects. These and other intriguing observations now require additional novel, exquisitely selective, non-peptide ligands as research tools to address many questions of fundamental importance concerning function of the mu, delta and kappa opioid receptor subtypes.

STIMULANT ABUSE TREATMENT AND PREVENTION

The abuse of cocaine is widely recognized to be an extremely serious health and social problem of epidemic proportions for which there is no effective treatment. The extent of the problem is evident from the approximate 760 metric tons of illicit cocaine production valued at about \$20 billion at average 1999 U.S. kilogram wholesale prices. While cocaine production and abuse has stabilized at a very high level, illicit production of methamphetamine in the U.S. is growing almost exponentially with about 7200 individual clandestine methamphetamine laboratories (*20 per day*) closed by Federal, State and local authorities in 1999. Cocaine acts principally by inhibiting the ability of the DAT to return synaptic dopamine to storage vesicles thus increasing synaptic dopamine and mesolimbic dopaminergic transmission leading to the reinforcing effects of cocaine. In the case of methamphetamine, this drug is a substrate for the DAT and when transported into the storage vesicles causes diffusion of dopamine into the synapse and subsequent elevation of dopaminergic transmission. We adapted the approach of design and development of slowly dissociating agents that block the actions of cocaine on the DAT with less intrinsic activity than cocaine (Rothman *et al.* 1989). Such drugs may also block the action of methamphetamine by inhibition of its transport into the storage vesicles. We identified

GBR12909 as our lead compound that showed the desired characteristics. Using *in vivo* microdialysis in the rat, we found that while systemically administered GBR12909 produces a modest elevation of intrasynaptic dopamine in the nucleus accumbens, it blocks the large elevation of dopamine by cocaine in a dose-related manner. This approach to treatment and prevention of cocaine abuse has been validated with our finding that GBR12909 prevents cocaine self-administration in rhesus monkeys trained to self-administer cocaine with no effect on normal behavior as measured by food maintained responding (Glowa *et al.* 1995). Studies with repeated administration of GBR12909 have shown sustained therapeutic effects on cocaine self-administration. For example, in a 12-day treatment study with GBR12909, cocaine self-administration was eliminated in monkeys with no effect on food intake. No evidence for the development of tolerance was observed. When the treatment drug was discontinued after 12 days, cocaine self-administration returned to the pretreatment level but could again be eliminated by renewed GBR 12909 treatment. Our PET studies in the baboon have subsequently shown that GBR12909 blocks the accumulation of [¹¹C]WIN35428 (a cocaine analog metabolically more stable than cocaine) on the DAT in a dose-related manner. In addition, doses of GBR12909 that prevent cocaine self-administration substantially occupy the DAT as shown by the displacement of [¹¹C]WIN35428 (Villemagne *et al.* 1999). We designed and synthesized a racemic 3-hydroxy-3-phenylpropyl derivative of GBR12909 with a binding and uptake inhibition profile nearly identical to that of GBR12909. Conversion of this material to its decanoate ester afforded an ultra long acting cocaine treatment agent in the rhesus monkey. *One dose* of this compound prevented cocaine self-administration in rhesus monkeys without effecting food maintained responding for nearly 30 days (Glowa *et al.* 1996). Recently, our *in vivo* microdialysis studies in the rat showed that two weeks after administration of this decanoate derivative, methamphetamine elevation of dopamine in the nucleus accumbens after acute administration is nearly eliminated. These data suggest that this strategy may be useful in the treatment of abuse of methamphetamine, as well as that of cocaine. We also synthesized and studied the enantiomers of the 3-hydroxy derivative and found nearly identical binding and reuptake inhibition. By contrast, when the hydroxy group was moved to the adjacent carbon atom to provide a 2-hydroxy derivative the *S*-enantiomer was substantially more active *in vitro* and *in vivo* than the *R*-enantiomer although both enantiomers eliminated cocaine self-administration in rhesus monkeys without effecting food intake. GBR12909 is now in Phase 1 clinical trials under the auspices of the National Institute on Drug Abuse. This compound and the enantiomers of the 2- and 3-hydroxy compounds provide an array of potential candidates for human treatment. In addition, the hydroxy compounds can be converted to decanoate ester derivatives to provide long acting drugs possibly with some having different and more favorable side effect profiles than those of the racemic 2-hydroxy material.

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