Perspective

Selective Opioid Receptor Agonists and Antagonists: Research Tools and Potential Therapeutic Agents

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Received September 25, 1989

The powerful pain relieving and mood altering effects of opium have been known for centuries, and for almost that long scientists have sought to understand the pharmacology of the active constituents of opium, the opiates. Over the years, there have been certain events that have dramatically influenced the course of opiate research. Such was the case with the initial proposals, which were based on whole animal pharmacology, for the existence of multiple "opioid" receptors.[1-4] These caught the attention of many, in part, because they gave new life to the idea of developing opioid analgesics that would be free of the highly undesirable side effects of the opiates, such as physical dependence and abuse. There was an infusion of new scientific talent into this area of research with which our knowledge of the opioid receptor system was able to progress rapidly. At this time, three distinct opioid receptors have been well characterized, the μ, κ, and δ,[5-9] and evidence continues to accumulate for the existence of subtypes of these receptors. In addition, endogenous ligands for the opioid receptors have been identified, and much is now known concerning their individual pharmacological activities.[10-12]

These discoveries have been impressive, and opioid research continues to offer considerable promise for the future development of new therapeutic agents. Opioid receptors and their endogenous ligands are now known to have important physiological functions in addition to the modulation of pain experience. Thus, the discovery of opioids with alternative therapeutic properties is possible.

In an attempt to characterize and understand the different opioid receptors and the pharmacologies of their endogenous ligands, many different research strategies have been successfully employed. One approach, the identification and characterization of selective opioid agonists and antagonists, has been particularly instrumental in the advancement of multiple opioid receptor knowledge. The intent of this Perspective is to review the impact that this approach has had, any continues to have, on opioid receptor research. Because of the enormous amount of research that could be included within the scope of this topic, closer attention will be directed to the selective opioid antagonists. This appears justifiable because of the importance that differential antagonism has had for advancing new pharmacological concepts.

Opioid Receptor Selectivity

When an agent is described as being selective, it is meant to indicate that it has higher affinity for a particular receptor than it has for other receptors. The experimental criteria used to define selectivity for the various opioid receptors have evolved over the last several years, and it is important to note that these criteria are continually being refined as our knowledge of the opioid receptor system increases. At this time there is no "gold standard" for defining opioid receptor selectivity, and realistically only when this system is fully characterized will such criteria be established. Already compounds have been identified that have opioid pharmacologies not readily categorized into the framework of the μ, κ, and δ receptors. These anomalous compounds may be the tools through which further differentiation of opioid receptor subtypes will be achieved.

Radioligand binding assays for measuring affinities at the μ, κ, and δ opioid receptors are available and a variety of different ligands are used.[8,13-17] Some assays involve the use of highly selective ligands to label a specific receptor, whereas other assays use nonselective ligands in the presence of other selective opioids to define binding to a particular receptor. There also exists several different bioassays using isolated tissues that can quantify the opioid agonist and antagonist properties of an agent.[8,13] Results from various binding assays and bioassays have been compared with in vivo opioid activities, and correlations with various pharmacological effects or the differential antagonism of these effects have been established.

Agents considered to be selective for an opioid receptor can have widely varying degrees of selectivity and currently there are no specific opioid agents known. Obviously, the more selective the agonist or antagonist employed in a study, the more firm are conclusions on opioid pharmacology. However, it is important to recognize that pharmacologic studies of agents with only a limited degree of

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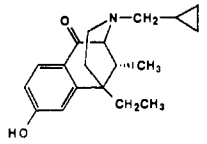
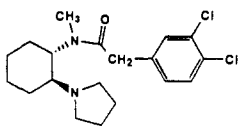
selectivity have led to important discoveries. For example, Martin and colleagues first proposed the existence of the κ receptor as distinct from the μ (morphine) receptor through careful comparison of the actions of morphine, ketocyclazocine (KC), and ethylketocyclazocine (EKC).[5,6]



ETHYLKETOCYCLAZOCINE



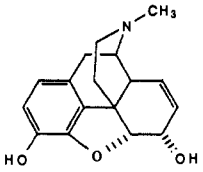
U-50,488

This was possible even though KC and EKC are now considered to be rather nonselective κ agonists. Furthermore, the existence of the δ receptor was first proposed by Kosterlitz and co-workers through comparisons of morphine and the enkephalins in bioassays and binding assays in which the enkephalins have only marginal selectivities.[7]

The ideal ligand to characterize a receptor would display high selectivity in a variety of in vitro and in vivo assays. Often this is not the case, and the degree of selectivity of a particular compound can vary dramatically from one assay to another, especially from in vitro to in vivo conditions. Considerable caution must be used when inferring in vivo selectivity based upon in vitro data. In addition, selectivity can vary between species, tests, routes of administration, time after administration, and on the concentration or dose of the drug used. Because of these factors, discrepancies as to the actual selectivity of an agent can sometimes exist in the literature. Therefore, careful attention needs to be given to how the selectivity of an agonist or antagonist has been characterized.

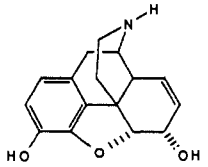
Selective Opioid Agonists

μ Receptor. There are a number of routinely used agonists with diverse structures that have good selectivity for the μ receptor. Many of these agents were discovered long before the concept of multiple opioid receptors was proposed; morphine is still considered to be the prototypic

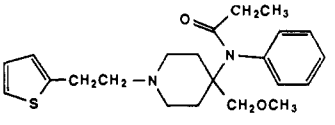


MORPHINE

μ receptor agonist. The affinities of morphine for the κ and δ receptors are sufficiently low that they are seldom of consequence in the pharmacological actions of morphine.[8,18] Normorphine has high μ receptor selectivity, and because of its ease of removal from the tissue it is often used to activate μ receptors in isolated tissue bioassays.[8] Sufentanil is another potent opioid with high affinity and selectivity for the μ receptor.[8,16,17]



NORMORPHINE



SUFENTANIL

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The agonists with possibly the highest selectivity for the μ receptor have been discovered through structural modification of the enkephalins. Of the hundreds of opioid peptides synthesized, DAGO, which is also referred to as DAMGO (Tyr-D-Ala-Gly-MePhe-NH$(CH_2)_2$OH), is considered by many to be the most selective for the μ receptor.[19,20] DAGO is highly potent and can be used in vivo, although it crosses the blood–brain barrier (BBB) poorly. It has been useful in the characterization of μ-mediated receptor pharmacology particularly when differentiation from δ-mediated activity is being sought.

PLO17 (Tyr-Pro-*N*-MePhe-D-Pro-NH_2) is a μ agonist that has proven useful for studying peripheral μ receptor effects.[21,22] Recently, DALDA (Tyr-D-Arg-Phe-Lys-NH_2) has been found to have very high selectivity for μ receptors and does not appear to cross the BBB to any significant extent.[23]

κ Receptor. Since Martin's original characterization of the κ receptor,[5,6] the discovery of κ selective agonists was actively sought by several research groups. Compounds with κ receptor agonist potencies and selectivities greater than that of EKC were soon discovered, including bremazocine,[24] tifluadom,[25] and Mr 2034.[26,27] These drugs have been widely used to study the pharmacology of the κ receptor. However, in spite of their κ selective agonist properties, they bind with high affinities to all three opioid receptors, and in carefully designed studies, their other receptor-mediated actions can be identified. Dynorphin (Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp-Asp-Asn-Gln; DYN_{1-17}), the likely endogenous ligand for the κ receptor,[28] has a limited selectivity for the κ receptor, as do the analogues DYN_{1-9} and DYN_{1-13}.[29] Use of these agents to study κ receptor effects is further complicated because they produce spinal agonist effects that are not antagonized by opioid antagonists and therefore are not believed to be opioid effects.[30-33]

U-50,488, discovered by scientists at the Upjohn Research Laboratories, was the first κ agonist to bind selectively to the κ receptor.[34-40] Because of this, U-50,488 has

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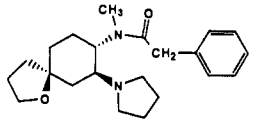
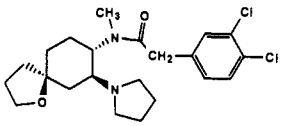
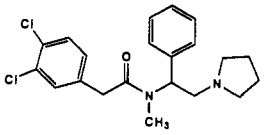
been widely used to delineate κ opioid receptor activities and more than 300 citations to its use can be found. Further structural modification of this molecule led to the discovery of even more selective agents, U-62,066[41] (spiradoline) and U-69,593.[42] [^{3}H]-U-69,593 is commercially available and has been used to selectively label the κ receptor.[42] Recently, the discovery of other highly selective κ agonists PD117302[43-46] and ICI199441,[47] which are structurally related to U-50,488, have been reported.



U-69,593



U-62,066



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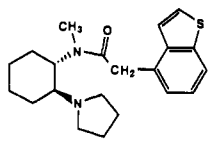
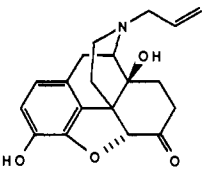


PD117302

δ Receptor. The δ agonists with the highest selectivity all have peptide, enkephalin-derived, structures. Hundreds of enkephalin analogues have been synthesized and studied for δ receptor selectivity. Early on, DADL (D-Ala^2,D-Leu^5-enkephalin) was found to have improved biological stability and some selectivity (3–10-fold) for the δ receptor compared to the μ receptor.[48] Even though its selectivity is limited, DADL has been widely used to study δ receptor pharmacology. Subsequently, DSLET (H-Tyr-D-Ser-Gly-Phe-Leu-Thr) was discovered and found to have at least a 20–600-fold selectivity, depending on the assay, for δ over μ and κ receptors.[49] The synthesis of more rigid analogues of the enkephalins, the bispenicillamide derivatives, led to the discovery of the highly selective δ agonists, DPLPE and DPDPE (D-Pen^2,L-Pen^5- and D-Pen^2,D-Pen^5-enkephalin).[50] In binding assays, the affinities of these agents for δ over μ receptors are 175- and 340-fold, respectively, whereas their selectivities for δ versus κ receptors are greater than 5000-fold. In smooth muscle bioassays, their selectivities for δ over μ receptors are 1000- and 3000-fold.[51] These agents, particularly DPDPE, are being used successfully for the characterization of δ receptor activities. They are active in vivo but are rarely used systemically because of very poor penetration of the BBB.

Selective Opioid Antagonists

Naloxone was the first pharmacologically pure opioid antagonist identified, and it would be difficult to overstate the importance of its discovery. It is considered to be a "universal" opioid antagonist, and even today an action of an agonist is characterized as opioid-receptor mediated only if its effects are naloxone reversible.[13] The unnatural



NALOXONE

(+)-isomer of naloxone has been shown[52] to have 10 000-fold less affinity for opioid receptors than the (−)-isomer and is inactive[53] as a narcotic antagonist. The total synthesis and availability[54] of the former has provided a useful control for the detection of opioid receptor mediated effects by naloxone.[55] Compared to other neuronal receptors, relatively few antagonists for opioid receptors are known.[56,57] With the opioid receptors, compounds that bind generally have some degree of agonist activity at least for one of the receptors, and numerous full and partial agonists are known. The number of agents with selective antagonist properties is very small; however, in addition to these, compounds have been discovered that will selectively and irreversibly bind to individual opioid receptors. These noncompetitive ligands, if over time they inactivate opioid receptors, often have the potential to be used in a similar way to long-lasting antagonists. This is not to say that covalent binding necessarily leads to irreversible antagonism. In fact, one irreversible opioid agonist has been described.[58]

μ Receptor. While naloxone binds to all three opioid receptors, it has highest affinity for the μ receptor.[8] With careful dose selection antagonism of μ receptors can be achieved without significant effects on κ and δ receptor activities. Determination of naloxone pA_2 values is con-

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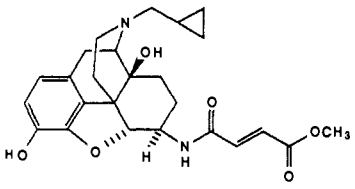
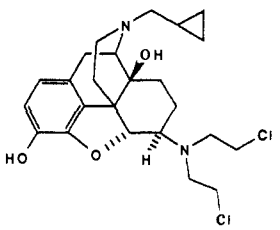
sidered to be one means of classifying receptor activation by an opioid agonist.[18,39,59,60]

β-Funaltrexamine (β-FNA) was the first agent characterized that would, in a highly selective manner, antagonize μ receptors.[61-63] It was discovered by Portoghese and Takemori, who were using a site-directed alkylating agent approach to develop selective opioid antagonists.[64-67] As such, β-FNA irreversibly, and selectively, binds to μ receptors. Previously, this strategy led to the discovery of β-chlornaltrexamine (β-CNA), a compound that will,



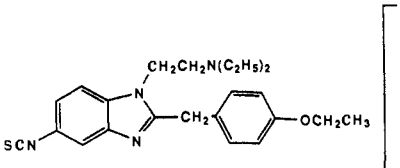
β -FNA



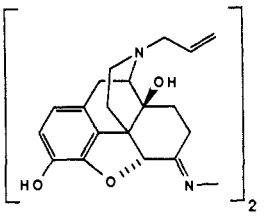
β -CNA

nonselectively, alkylate all three opioid receptors.[68,69] When β-CNA is used in conjunction with a selective agonist, tissues depleted of all but a single opioid receptor (selective protection) can be prepared for pharmacological study.[65,66] Following the discovery of β-CNA, Portoghese and Takemori speculated that selective binding to an opioid receptor might be achievable if a less reactive electrophilic group was attached to the opioid ligand. This led them to the synthesis and evaluation of β-FNA. Interestingly, β-FNA initially binds reversibly with high affinity to all three opioid receptors.[70-73] Its ability to alkylate only μ receptors demonstrates the subtle but important structural differences that exist between the different opioid receptors, as do other aspects of the structure–activity relationships (SARs) of this series of compounds.[74] β-FNA does have agonist activity that is short lived, reversible, and appears to be κ receptor mediated.[62] Consequently, the selective μ receptor antagonist activity of β-FNA occurs only under irreversible conditions. The selective antagonist properties of β-FNA in various in vitro and in vivo situations, originally well documented by its discoverers, have been confirmed and further described in other laboratories.[75-79] Pretreatment with β-FNA in various isolated tissue assays, selectively depleting the μ receptor population, significantly improves these assays for characterizing non-μ properties of opioid ligands.[80-84] Such studies have also uncovered evidence suggestive of new opioid receptor subtypes.[74,79,85-89] β-FNA will cross the BBB and selective antagonism of μ activities can be seen following systemic administration, a property that has further increased the usefulness of this agent. Its use as a pharmacological tool has greatly facilitated our understanding of the opioid receptors and the pharmacology of the molecules that bind to them.[18,78,79,90-93]

Other researchers have been exploring different designs for the synthesis of selective inhibitors of opioid receptors. Using an amino-substituted analogue of the highly potent μ agonist etonitazene, Rice and co-workers were able to prepare a highly reactive and selective acylator of μ receptors, BIT.[94] The highly reactive nature of BIT enables



BIT



NALOXONAZINE

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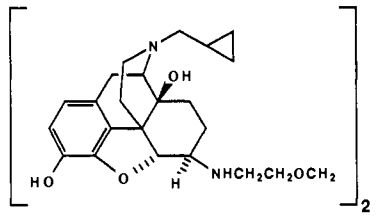
it to deplete membranes of μ binding sites, and as such, it has proven to be useful in the further biochemical characterization of the opioid receptors.[95-98]

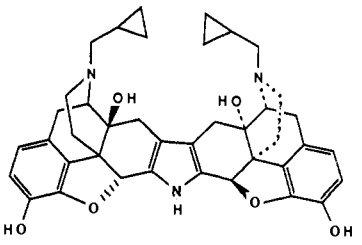
Another compound with apparent selective, long lasting (>24 h), μ receptor antagonist properties is naloxonazine, a C-6 azine-bridged dimer of naloxone.[99] The selectivity of this compound has been primarily documented by radioligand binding assays, and it has been characterized as a selective antagonist at μ_1, a putative μ receptor subtype.[100] It has antagonist activity following systemic administration and has been used in several pharmacological studies in an attempt to document the relevancy of the μ_1 binding site;[100-102] however, its in vivo antagonist properties against prototypic μ, κ, and δ agonists have not been fully characterized. The mechanism by which it produces the long-lasting effects remains to be clarified. There are conflicting reports as to the importance of covalent binding.[103,104] A very recent report questions the metabolic stability of naloxonazene and suggests the involvement of a hydrolytic cleavage product and membrane phosphatides in its persistent effects.[105]

Recently, D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Pen-Thr-NH_2 (CTP) has been shown to be a selective, reversible antagonist of μ receptors in isolated smooth muscle and radioligand binding assays.[22,106] It can be used in vivo but does not cross the BBB. This property has been used to its advantage to evaluate peripherally mediated μ receptor activities.[22,107]

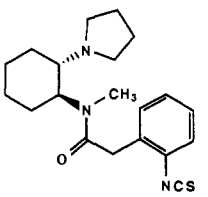
κ Receptor. Selective antagonists for the κ receptor have been actively sought for years but only recently have agents with good selectivity been discovered. Early references that cite Mr 2266 and also WIN 44,441 (quadazocine) as being κ antagonists, implying selectivity, are misleading.[8,108] The first antagonist documented to have a significant degree of selectivity for κ over μ and δ receptors was TENA.[109] Its antagonist activities were determined in isolated tissue bioassays, but only a modest degree of selectivity was observed, and there are no reports of its use in vivo. The design of TENA for possible selective opioid antagonist activity evolved from a concept that Portoghese and Takemori have termed the "bivalent ligand" approach.[110] Further application of this approach led them to the recent discovery of nor-BNI, a highly potent opioid antagonist with good selectivity for the κ receptor.[111-113] Its selective antagonist properties have been demonstrated in smooth muscle bioassays, radioligand binding assays, and whole animal studies.[114] It is weakly active following systemic administration; however, selective blockade of κ-mediated antinociception[115] and diuresis[116] has been achieved. Other researchers using smooth muscle assays further quantified nor-BNI as 400-fold selective for κ versus μ receptors and 250-fold for κ versus δ receptors;[117] however, they found only a limited selectivity for κ versus μ receptors in vivo. The reasons for the apparent differences observed in vivo appear to relate to the unusually long time following nor-BNI administration to peak κ selective antagonist effect.



TENA



nor-BNI



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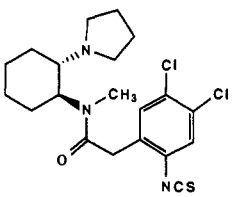

UPHIT

Very recently, the discovery of the first site-directed, irreversible inhibitors of κ receptors, compound 1[118] and UPHIT,[119] were reported. Rice and associates designed these acylators of κ receptors through structural modifications of the U-50,488 molecule. In radioligand binding experiments, appropriate concentrations of compound 1 will inhibit binding to κ receptors by 90% while having no significant effect on μ or δ receptor binding. Unlike compound 1, UPHIT will selectively acylate κ receptors fol-

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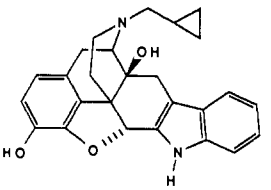
lowing in vivo (icv) administration. Surprisingly, both compound 1 and UPHIT fail to bind to all receptors previously thought to be κ,[118] possibly supporting earlier proposals for heterogeneity of κ receptors.[120,121] Indeed, further studies with UPHIT have now led to the suggestion of four κ binding sites in guinea pig brain.[122]

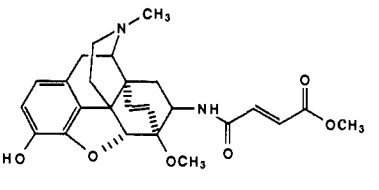
δ Receptor. The enkephalin analogue ICI 154129 was the first antagonist discovered with substantial selectivity for the δ receptor.[123] Its 30-fold selectivity for the δ versus other opioid receptors was impressive, but its low potency limited its utility. Subsequently, another enkephalin analogue, ICI 174864 (diallyl-Tyr-Aib-Aib-Phe-Leu), was found to be a much more potent and selective antagonist of δ receptor activation.[124,125] In the mouse vas deferens smooth muscle assay, the δ receptor antagonist potency of ICI 174864 is at least 200 times greater than its μ and κ antagonist potencies. ICI 174864 also has been reported to have opioid agonist activity at high concentrations, possibly δ receptor mediated,[126] and it produces opioid analgesic effects at high doses following icv administration.[127] In spite of these caveats, at appropriate doses, ICI 174864 will selectively block δ receptors in a variety of different situations. These properties have made it a very useful tool for studying δ opioid receptor mediated effects.

Very recently, the discovery of the first non-peptide, δ antagonist was reported, naltrindole (NTI).[128] NTI is a naltrexone analogue with a conformationally restricted aromatic system fused to its C_{6-7} carbons. It is speculated that NTI is a bivalent ligand and that the two aromatic rings align in a similar manner to the aromatic rings of the enkephalins when bound to δ receptors. In smooth muscle assays, NTI has a selectivity of approximately 150-fold for δ versus μ receptors and 280-fold for δ versus κ receptors. As a δ receptor antagonist it has a potency 80 times that of naloxone and 300 times that of ICI 174864.[129] Its δ receptor selectivity has also been demonstrated in vivo and it is active following systemic administration. Interestingly the *N*-methyl analogue of NTI has opioid agonist activity that appears to be mediated through δ receptors.[128]

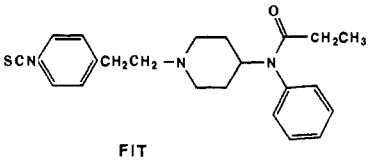
Selective irreversible inhibitors of δ receptors are also known. FIT, a fentanyl isothiocyanate derivative and FAO, a fumaramido oripavine derivative, were the first such agents discovered.[94] They both selectively covalently bind to δ receptors, with FIT being the more potent of the two. Because FIT is an analogue of the highly selective μ agonist, fentanyl, its selective ability to acylate δ receptors was quite surprising and again underscores the subtle structural differences between opioid receptors mentioned



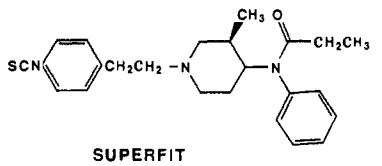
NTI



FAO



FIT



SUPERFIT

above. FIT, however, has been shown to bind reversibly to μ receptors.[130] These findings further demonstrate how difficult it can be to predict the selectivity of a certain molecule.[131,132] As with other agents of this type, FIT is a selective inhibitor of δ binding only under irreversible conditions.[133] Further structural modification of the FIT molecule led Rice and associates to the discovery of SUPERFIT, (+)-*cis*-methylfentanyl isothiocyanate.[134] Like FIT, SUPERFIT also selectively acylates δ receptors but with a potency 5–10 times that of FIT. As indicated, SUPERFIT is a single optical isomer and is 50 times more potent than its (−)-isomer as an acylator of δ receptors. The (+)-trans isomer of SUPERFIT has recently been synthesized and is also highly potent in producing wash-resistant inhibition of δ receptors.[135] FIT and SUPERFIT are being used to facilitate understanding of the opioid receptors at the molecular level[136–140] including the identification and characterization of the actual δ receptor binding protein.[141,142] The increased potency of SUPERFIT and the resulting reduction in nonspecific binding

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have made it particularly valuable in such studies.

Importance as Pharmacological Probes

With the realization that the actions of the opioids were mediated through multiple receptors, medicinal chemists and pharmacologists have sought selective agonists and antagonists as a means to identify the various effects mediated by the individual receptors. Even though our current understanding of the opioid receptor system is limited, it is already evident that this approach has proven worthwhile. In fact, our current knowledge of the functions of the μ, κ, and δ receptors appears directly related to the selective agents available for study.

A clear goal for many of those involved in opioid research has been the discovery of a safe, and highly efficacious, analgesic. Thus, the primary focus has been to understand how the opioids modulate pain perception and to identify which opioid receptors mediate their analgesic responses. Studies using agents of limited selectivity suggested that activation of κ receptors led to an analgesic response without respiratory depression, physical dependence, and drug reinforcing properties. Thus, the development of a κ agonist for analgesia use looked very promising. However, these earlier agents produced a characteristic dysphoric effect (referred to as psychotomimetic[143]) that precluded their use. Many have sought to identify the receptor through which these dysphoric effects are produced. The involvement of either the κ opioid,[38,144,145] the phencyclidine[146] (possibly Martin's original σ opioid receptor[147]), and the σ haloperidol sensitive,[148] non-opioid receptors have been proposed. The discovery of the highly selective κ agonists allowed for the direct evaluation of κ-mediated pharmacology in man. In a very recent abstract, spiradoline (U-62,066) has been reported to produce sedative and psychotomimetic effects in humans. These effects were antagonized by high doses of naloxone.[149]

Since the discovery of the δ receptor, researchers have been attempting to determine its role in mediating antinociception. Early pharmacological studies probing for such activity gave conflicting results,[49,150] which were possibly due to the limited selectivity of the agents under investigation. The discovery of highly selective δ agonists and antagonists have made more definitive studies possible. By comparing the antinociceptive activities of DPDPE, DAGO, and morphine, as well as the differential antagonisms of their effects with β-FNA and ICI 174864, δ-receptor-mediated antinociceptive activity has been demonstrated.[151-153] Of course, it is not known whether this will actually translate to clinical analgesia, and if so, whether a δ agonist will have therapeutic advantages over current analgesic therapies. These questions will remain unanswered until a selective δ agonist suitable for clinical evaluation is discovered.

While pain modulation is the best understood function of the opioid receptors, it likely represents only a small portion of the important physiological events that they mediate. Because of the widespread clinical use of morphine and other μ-selective opiates, the involvement of μ receptors in a wide variety of other functions has been known for a long time. These include euphoria, respiratory depression, tolerance, physical dependence, GI transit, and several others. Studies with the recently available, highly selective agonists and antagonists now suggest the possible existence of μ receptor subtypes,[22,151,154-156] indicating that all of these effects may not be produced through the same receptor. This gives hope and direction for the development of new and better therapeutic agents through selective μ receptor activation.

The discovery of highly selective and potent κ agonists has led to the delineation of several κ-receptor-mediated effects, including diuresis[157,158] (through inhibition of release of the antidiuretic hormone vasopressin), sedation, physical dependence,[159] and others.[160] κ agonists also produce distinct discriminative effects in laboratory animals.[38,161] The relatively recent discovery of the selective κ antagonist, nor-BNI, provides an additional means to study κ-mediated function. nor-BNI has been reported to improve the outcome in traumatic spinal cord injury,[162] and other studies with nor-BNI in various therapeutically important situations are likely underway. Selective irreversible κ ligands are now available and will be used to further characterize κ receptors.

Of the three opioid receptors, the functions of the δ receptor are the least understood. In the past it has been very difficult to distinguish δ-receptor-mediated pharmacology. Even now, most of the selective δ ligands cross the BBB only poorly, and this limits their usefulness as pharmacological probes. However, apparent antinociceptive activities of δ agonists have now been identified, and there is evidence suggesting an involvement of δ receptors in the regulation of GI transit and modulation of certain μ receptor activities. The recent discovery of the highly potent and selective δ antagonist NTI provides researchers with a potentially important new tool for studying δ receptor function.

While our knowledge of the opioid receptor system has increased tremendously, this new information has not yet led to the development of a major new therapeutic agent. It may be that many of the discoveries are too recent for them to have made their full impact, or that additional understanding is needed before the means to achieve the requisite pharmacological selectivity is uncovered. Although several selective agonists and antagonists are now

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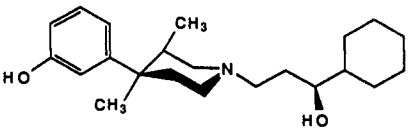
available for study, it is clear that others will have to be discovered before the activities of the μ, κ, and δ receptors can be fully explored. It also seems likely that the opioid receptor system is more complex than it is now described. Much opioid-receptor-mediated pharmacology cannot be associated with a particular receptor, and some of this pharmacology could have considerable therapeutic potential, including effects on consumption,[163,164] sexual behavior,[165] seizures,[166,167] shock,[168] immune function,[12,169-171] and mood.[172] Many investigators are attempting to characterize and assess the importance of these effects. There is also a new emphasis on understanding the importance of opioid regulation of gastrointestinal motility and secretion and identifying areas for possible drug development.[173-179]

Much attention is being directed to the further characterization of these other opioid pharmacologies and assessing their therapeutic importance. As new selective agonists and antagonists, characterized by the criteria of today, are discovered, they are used as pharmacological probes. Another productive approach is to use in vivo assays to establish SARs for a particular effect and then relate these findings to the relative opioid receptor affinities. Recently, we used this strategy to discover an opioid antagonist with remarkable appetite suppressant properties, LY255582.[180] The SARs observed for this effect did



LY255582

not correlate with affinities or with in vivo measures of antagonist potencies for either the μ, κ, or δ receptors.[181] This finding might suggest the involvement of other opioid receptors in the regulation of appetite or a combination of opioid receptor effects to obtain the desired pharmacology.

Other investigators are turning to the biochemical characterization and purification of opioid receptors to understand opioid receptor multiplicity and function.[182-185] These efforts also have relied heavily on the use of selective opioid receptor ligands. Although opioid receptors are proving to be very difficult to work with, subunits to the μ and δ receptors have been purified and an opioid-binding protein has been recently characterized by cDNA cloning.[186]

The further differentiation of opioid receptors appears to be very important for the development of new therapeutic agents that act at opioid receptors. Because there is already a considerable amount of evidence suggesting the existence of subtypes of the known opioid receptors, the future of opioid research for drug development would appear to be very promising. Our current knowledge of the opioid receptor system has been highly dependent upon the development of selective agonists and antagonists, and the discovery of other new agents will continue to be critical for further unravelling of this very complex system. Only through the use of selective ligands will the physiological functions of the various opioid receptors and therapeutic implications of these be fully understood.

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