Themed Issue: Drug Addiction - From Basic Research to Therapies
Guest Editors - Rao Rapaka and Wolfgang Sadée

Molecular Recognition of Opioid Receptor Ligands

Submitted: June 17, 2005; Accepted: December 15, 2005; Published: March, 10, 2006

Brian E. Kane,[1] Bengt Svensson,[1] and David M. Ferguson[1]

[1]University of Minnesota, College of Pharmacy, Department of Medicinal Chemistry, Minneapolis, MN 55455

ABSTRACT

The cloning of the opioid receptors and subsequent use of recombinant DNA technology have led to many new insights into ligand binding. Instead of focusing on the structural features that lead to increased affinity and selectivity, researchers are now able to focus on why these features are important. Site-directed mutagenesis and chimeric data have often been at the forefront in answering these questions. Herein, we survey pharmacophores of several opioid ligands in an effort to understand the structural requirements for ligand binding and selectivity. Models are presented and compared to illustrate key sites of recognition for both opiate and nonopiate ligands. The results indicate that different ligand classes may recognize different sites within the receptor, suggesting that multiple epitopes may exist for ligand binding and selectivity.

KEYWORDS: Opioid, structure-function, pharmacophore, mutagenesis, chimeric

INTRODUCTION

Over the years, a great amount of effort has been devoted to the development of structural models that predict ligand binding and selectivity for the μ, δ, and κ opioid receptors. In the absence of crystallographic data, indirect methods, which include site-directed mutagenesis, chimeric studies, the substituted cysteine accessibility method, and affinity labeling studies, have been instrumental in locating key contacts for molecular recognition. One of the most informative methods has been the engineering of chimeric receptors. By interchanging sequences of the μ, δ, and κ receptors, researchers have identified regions of the receptor responsible for discriminating differences between peptide and nonpeptide recognition, as well as regions necessary for selectivity.[1-4] For example, chimeric studies have shown that the second extracellular loop (EL-2) of the κ receptor is essential for the activity of the selective peptide agonist dynorphin A (Dyn A).[5,6] When EL-2 from μ or δ was inserted into the κ sequence, Dyn A lost activity. Conversely, when EL-2 from κ was inserted into μ or δ sequences, Dyn A gained activity.

Whereas chimeric studies generally paint a broad picture of what regions of the receptor may be important for binding, site-directed mutagenesis studies implicate individual residues. Thus, results often indicate how ligand recognition may be occurring at the molecular level. By the time opioid receptors were cloned, such studies were commonly used in the study of other receptor systems.[7,8] Important for the opioid receptor family were those on the β-adrenergic receptor, another G-protein coupled receptor (GPCR).[9] This GPCR binds epinephrine and numerous catecholamine analogs. Since opiates and opioid peptides share common structural features with epinephrine (Figure 1), it was suggested that they share similar interactions for their respective receptors.

One such interaction involves an aspartate residue in transmembrane helix III. This aspartate (Asp III:08, see Figure 2 for an explanation of the nomenclature) is conserved among all biogenic amine receptor families, including the β-adrenergic and opioid receptors.[10] When this residue in the β-adrenergic receptor was mutated to its neutral isostere asparagine (Asn), a large decrease in epinephrine binding was seen.[11] This suggested that a salt bridge between the amine of epinephrine and the carboxylate of the Asp had been disrupted. Since opioid ligands also possess an amine, a homologous interaction was proposed. When Asp III:08 in the μ receptor was mutated to Asn, several opioid receptor ligands did not bind, indicating a salt bridge.[1]

Further insight on opioid ligand recognition was realized by analyzing similar results from the β-adrenergic receptor system. For example, site-directed mutagenesis results suggested that conserved serine residues (V:09 and V:12) were hydrogen bonding to epinephrine's catechol moiety.[12] Since the structurally similar phenolic group is often essential for opiate and opioid activity,[13] it was believed that the formation of a hydrogen bond might be important in the opioid receptor family as well. However, since opioid receptors lack residues capable of forming hydrogen bonds at positions V:09 and V:12, an alternative site had to be considered. Presumably, this residue needed to be conserved, needed to be able to hydrogen-bond, and needed to be positioned at an appropriate distance from Asp III:08.

Corresponding Author: David M. Ferguson, Department of Medicinal Chemistry, University of Minnesota, 308 Harvard St SE, 8-101 Weaver-Densford Hall, Minneapolis, MN 55455. Tel: (612) 626-2601; Fax: (612) 624-0139; E-mail: ferguson@umn.edu

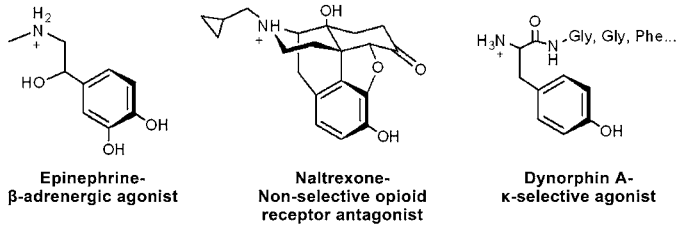

Figure 1. Epinephrine contains a *p*-hydroxyphenethylamine (tyramine) moiety, as do many opiates and opioid peptide ligands. Gly indicates glycine; Phe, phenylalanine.

A histidine located in TM VI (His VI:17) satisfied all these criteria. When this residue (in the μ receptor) was replaced with a residue incapable of forming hydrogen bonds (eg, alanine) decreases in binding occurred.[14] On the other hand, conservative mutations (ie, those that do not disrupt hydrogen bonding interactions, eg, histamine or glutamine to asparagine), had only minor effects on ligand binding.[15] From these results, it was suggested that His VI:17 hydrogen-bonds to the opioids' phenol.

Just as common structural moieties interact with conserved residues, uncommon moieties interact with variable residues within the receptor. This is best explained by the "message-address" concept.[16] In short, the message-address concept states that ligands contain different recognition elements that are responsible for their differential binding activities. Their shared, or universal, portion represents the "message," while their unique, or variable, portion represents the "address." For the opiates, the tyramine moiety (the amine and phenol) represents the message, while large substituents on the C ring represent the address (Figure 3).[17] The nonselective ligand naltrexone (NTX)[18] does not contain an address and thus is not selective. Meanwhile, the δ-selective ligand naltrindole[19] (NTI) contains indole moiety, and the κ-selective ligand 5-guanidinylnaltrindole[20] (gNTI) contains a guanidinyl moiety that acts as the address to confer selectivity.

It should be noted that the opiates are exceptional examples of the message-address theory, because both the message and address moieties are well defined. In contrast, nonopiates such as fentanyl[21] (μ-selective agonist), U50,488,[22] and U69,593[23] (κ-selective agonists) do not contain a traditional message and address. For these 2 ligand classes (the fentanyls and arylacetamides), docking studies predict a unique epitope that has minimal overlap with the opiate

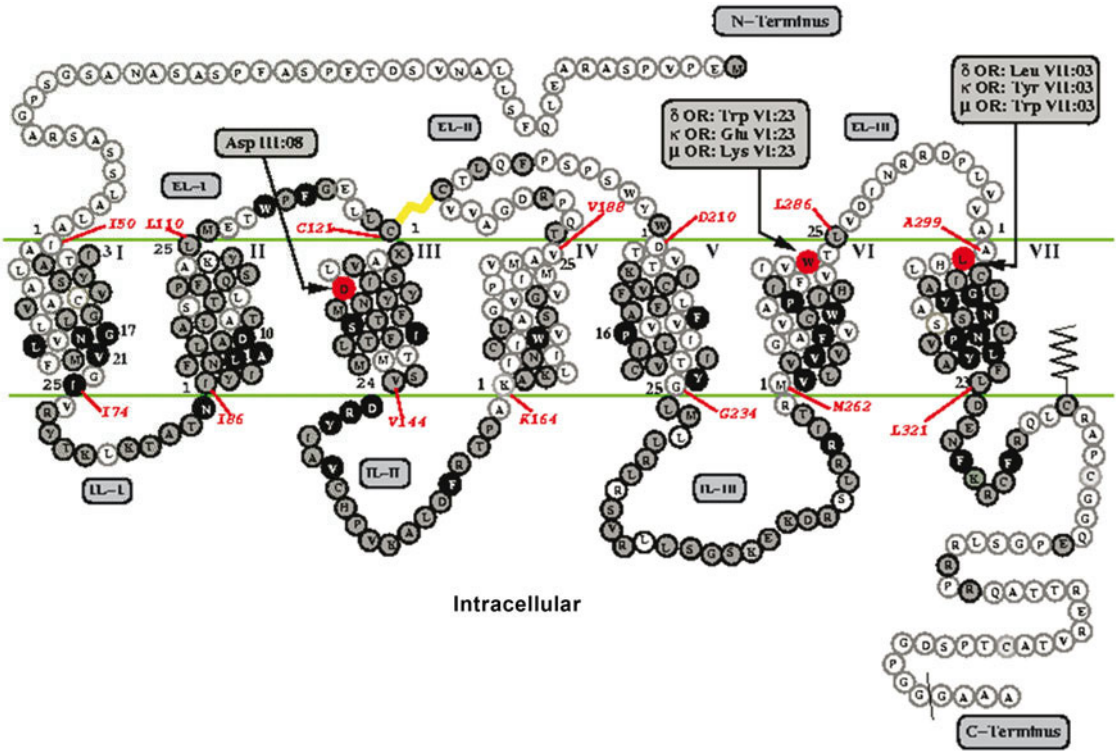

Figure 2. Serpentine model of the δ receptor. Circles contain the 1-letter code for the given amino acid. Green lines indicate the beginning and ends of the helices. The gray circles indicate the residues that are conserved among all 3 receptor types (μ, δ, and κ), while the black circles indicate the residues that are highly conserved among the rhodopsin subclass of G-protein coupled receptors. Each transmembrane (TM) region is indicated by a roman numeral. At the beginning and end of each helix there are Arabic numbers starting with 1 and ending at 25 (variable, depending on helix length). These numbers correspond to the position of the residue within the helix. For example, the Asp in TM III is denoted as III:08. Asp indicates aspartate; EL, extracellular loop; Glu, glutamine; IL, intracellular loop; Leu, leucine; Lys, lysine; OR, opioid receptor; Trp, tryptophan; Tyr, tyrosine.

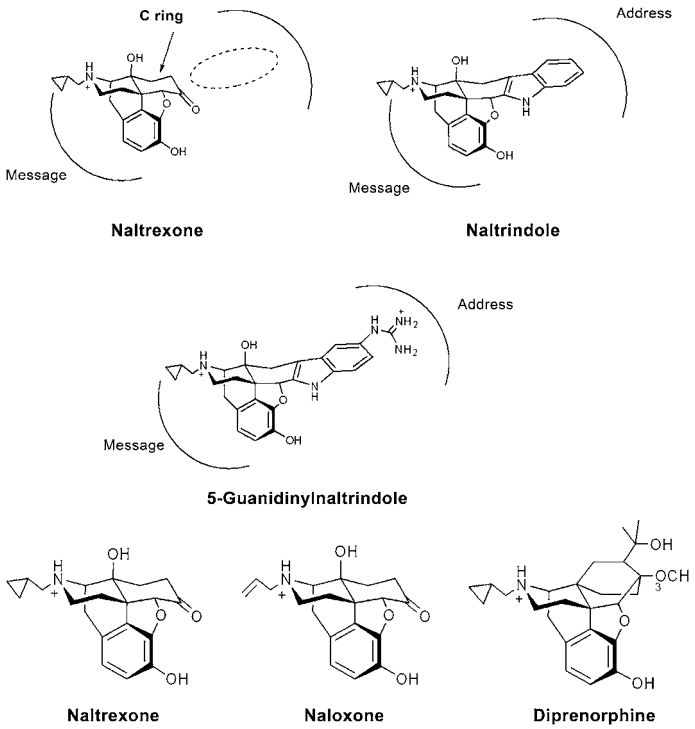

Figure 3. Structural representation of the "message-address" concept.

pharmacophore.[24-30] Thus, this review is broken up into sections based on ligand class. For each class, molecular recognition will be discussed in terms of how specific interactions assist ligand binding.

Opioid Receptors: Model Development

Before the molecular recognition of opioids is presented, a general discussion of opioid receptors must be undertaken. Because opioid receptors do not have a crystal structure, sequence analysis methods have been important in revealing many of the receptors' general characteristics. These methods, which include hydropathy plots and multiple sequence alignments, have classified the opioid receptors (μ, δ, and κ) into the class A GPCRs resembling rhodopsin.[31] These membrane-bound proteins contain an extracellular N-terminus, 7 transmembrane helices, and an intracellular C-terminus (Figure 2). The helices are bundled in a counterclockwise fashion and connected intra- and extracellularly by loops that vary in size and composition.

Also evident from Figure 2 is the high sequence homology between the 3 opioid receptor types (indicated by gray and black circles). The transmembrane helices show ~70% identity, and the loops show ~60% identity.[31] In both cases, however, sequence identity is dependent on the region of the receptor being analyzed. For example, while the intracellular loops share ~90% homology, the N-terminus, EL-2, EL-3, and the C-terminus share little to no homology. Likewise, TM helices II, III, and VII have ~75% identity, while helices IV, V, and VI share considerably less sequence identity.

Each receptor type has also been further characterized into subtypes. Pharmacological and radioligand studies point toward at least 2 variants for each receptor type, namely μ_1/μ_2, δ_1/δ_2, and κ_1/κ_2.[31] The δ_1 receptor, for example, has been characterized based on the ability of 7-benzylidenenaltrexone and the enkephalin analog DALCE to selectively antagonize the antinociceptive activity of DPDPE and DADLE.[32] Meanwhile, δ_2 is characterized based on the ability of naltriben and 5′-NTII (naltrindole isothiocyanate) to selectively antagonize deltorphin II and DSLET.[33] Since both receptor subtypes share the same amino acid sequence, the unique binding profiles have been speculated to be the result of different posttranslational modifications, distinct cellular localizations, and varying interactions with other associated proteins.[34] It remains largely unknown how these interactions contribute to the receptor's overall conformation. Thus, even as newer models (derived from the more recently acquired high-resolution crystal structure rhodopsin)[35,36] replace older bacteriorhodopsin-derived models, the connection between receptor conformation and resultant biological activity has not been resolved.[30,31,37-39]

Despite this fact, the development of pharmacophores for opioid receptor ligands has flourished. One approach to identifying these pharmacophores uses automated docking simulations (eg, the DOCK[40] suite of programs) to predict binding modes. Flexible ligands often produce a larger number of different but plausible binding site models. Thus, narrowing down the binding modes is often more difficult. In some instances, biophysical data are available to support one of the proposed pharmacophores or to reject the existence of another. Much of the time, such data do not exist and the selection of a binding mode is based on the researcher's interpretation. This can lead to pharmacophores that vary tremendously. Conversely, the models developed for the more rigid opiates are very similar to one another. These models are also supported by more extensive biophysical data. Since it is thought that nonopiates bind in a similar pocket, the more refined opiate models have often been the starting structure for docking studies for nonopiates. Thus, they will be discussed first to provide a basis for the other opioid receptor models.

Pharmacophoric Models

Nonselective Opiates

Naltrexone is a known as a universal opiate antagonist. It and the closely related nonselective opiates naloxone[41] and diprenorphine[42] all bind to the μ, δ, and κ receptors with very high affinity. Furthermore, these ligands share structural similarities that are thought to be the source of their activities. First, they contain an amine that is thought to be

protonated at physiological pH; second, they contain a phenolic ring. Together, these components form a tyramine or "message" moiety. Lastly, these opiates contain a hydrophobic portion. Approximately 50 years ago, Beckett and Casy proposed a crude receptor model that encompassed these 3 characteristics (Figure 4).[43]

Beckett and Casy's 3-point model was invaluable for explaining generic receptor-ligand interactions; however, it did not take into account the specific interactions responsible for ligand recognition. Since the success of drug discovery often lies in determining such details, transforming this model into a contemporary pharmacophore that could implicate individual residues was essential. As mentioned in the introduction, comparisons of opiates to the catecholamines suggested that binding occurs within conserved regions of the transmembrane helices, specifically using interactions between an amine and Asp III:08, and a phenol and His VI:17.[1,14] These interactions accounted for two thirds of the 3-point model, but the determination of the residues suggested to interact with the hydrophobic region still remained.

Given the relatively small nature of the nonselective opiates, it was presumed that a hydrophobic pocket might be found in close proximity to Asp III:08 and His VI:17. Sequence analysis revealed that a cluster of conserved hydrophobic residues existed approximately halfway down TM helices III to VI (Trp IV:10, Phe V:13, Phe VI:09, and Trp VI:13).[44] These residues were mutated in order to determine the role of aromatic transmembrane residues of the δ receptor in ligand binding.[45] Results suggested that the residues Trp V:10, Phe V:13, and Trp VI:13 might help form the putative hydrophobic pocket. Since these residues are conserved, it was suggested that these residues play a similar role in the μ and κ receptors as well. A summary of these interactions, using naltrexone as a representative ligand, is shown in Figure 5.

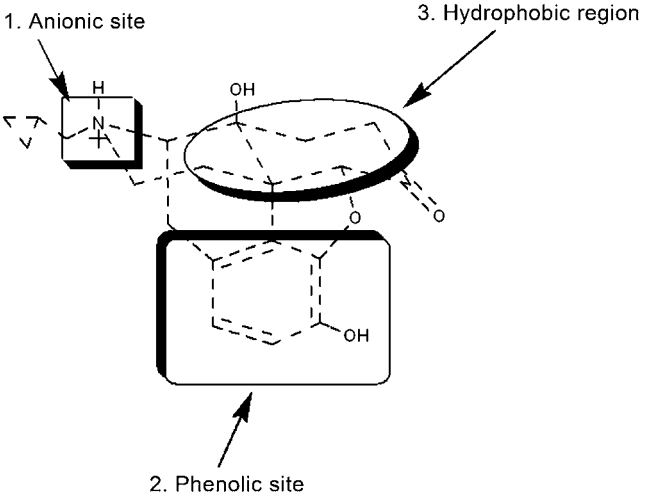

Figure 4. Above: Nonselective antagonists naltrexone, naloxone, and diprenorphine. Below: Beckett and Casy's 3-point receptor model shown with naltrexone as a representative ligand. It is suggested that the first 2 points of the model, the anionic and phenolic site, interact with the tyramine "message" moiety. The third point of the receptor model suggests that a hydrophobic region within the receptor stabilizes the remaining alkaloid scaffold, specifically rings C to E.

Selective Opiates

The 3-point model is well suited to describe the interactions of nonselective opiates. However, it does not account for interactions of the "address" moieties found in selective opiates like NTI and gNTI (Figure 3). For these ligands, an address locus containing variable residues is believed to be responsible for conferring selectivity. Experimental data support this hypothesis. Specifically, chimeric studies have revealed that the highly variable region from the top of TM VI to the C-terminus is required for high affinity binding of the κ-selective antagonist norbinaltorphimine (norBNI).[46] Additionally, chimeric studies have determined that the same region is implicated for δ-selective opiates.[4]

Once this region had been implicated as an "address" locus, it was hypothesized that individual residues within this region were responsible for conferring selectivity. In general, 2 techniques, alanine scanning and directed mutation, were applied to determine what residues, if any, were involved. The former method is a technique in which a side chain is individually and randomly mutated to alanine. It is hypothesized that if a large change in binding is observed, then the residue may be involved in ligand binding, thus warranting further investigation. Results from this method revealed that 3 residues near the top of TM VI and VII were

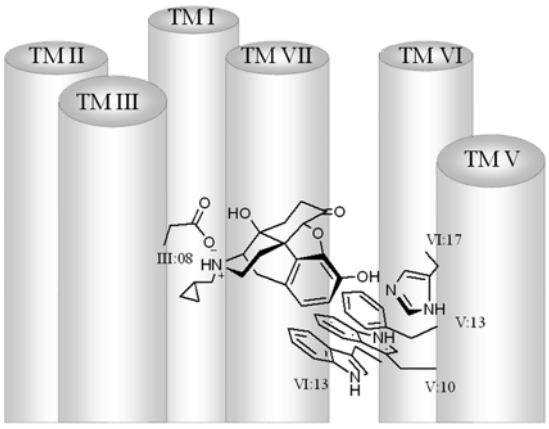

Figure 5. The pharmacophore of the nonselective opiate naltrexone. The tyramine "message" moiety forms a salt bridge with Asp III:08 and a hydrogen bond with His VI:17. Aromatic residues that stabilize the hydrophobic core include Trp V:10, Phe V:13, and Trp VI:13. Asp indicates aspartate; His, histidine; Phe, phenylalanine; TM, transmembrane; Trp, tryptophan.

implicated in binding to a wide variety of δ-selective ligands (these individual sites and their importance to ligand binding will be discussed in detail in the section concerning δ-selectivity).[47] The other method, directed mutation, uses molecular models to determine which residues to mutate. Under the assumption that the shared alkaloid scaffold of selective opiates binds in a similar manner as the nonselective opiates, the models suggested that there are 2 positions that are both unique and oriented appropriately for interaction with the ligands.[45] These 2 positions, VI:23 and VII:03, occupied by Glu297 and Tyr312 in κ, Trp284 and Leu300 in δ, and Lys303 and Trp318 in μ, became the targets for numerous site-directed mutagenesis studies.

Site-directed mutagenesis results from these mutants suggest that selectivity for opiates arises from the interplay of 2 distinct mechanisms, mutual attraction and steric exclusion. For mutual attraction, it is hypothesized that a ligand is attracted to a complimentary residue or group of residues within the receptor. The incurring stabilization, generally from charge neutralization or hydrophobic interactions, leads to increased binding. The second mechanism, steric exclusion, occurs when a residue or a group of residues does not allow a favorable or complimentary interaction (mutual attraction) to occur. As will be seen in the following sections, each opioid receptor type achieves selectivity by using these 2 mechanisms together.

Kappa-Selective Opiates

Ligand recognition of gNTI and norBNI[48] (Figure 6) has been studied extensively. One obvious difference between these κ-selective opiates and the nonselective opiates is the presence of a second basic moiety found in the "address." This basic moiety has been implicated in forming a salt bridge with a unique glutamate within the κ receptor, Glu VI:23.[20,49] Specifically, when Glu was mutated to a lysine (the homologous residue found in μ), a significant decrease in binding for norBNI and gNTI was observed. Meanwhile, no significant changes were observed for the nonspecific ligands naloxone (NLX) and naltrexone (NTX). Furthermore, when the homologous position in μ (K303) was mutated to glutamate (as to mimic the κ receptor), norBNI and gNTI's activity was comparable to that of wild-type κ.[20] Studies conducted in mutant

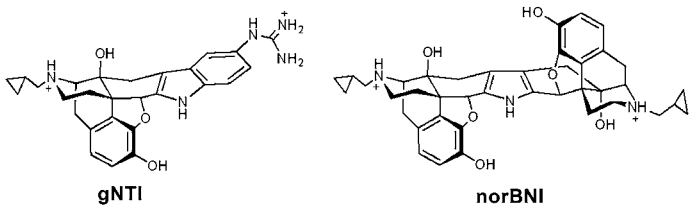

Figure 6. Kappa-selective opiates gNTI and norBNI. gNTI indicates 5-guanidinylnaltrindole; norBNI, norbinaltorphimine.

δ receptors also exhibited similar trends. For example, when Trp VI:23 was mutated to glutamate, activity for norBNI and gNTI was significantly enhanced.[20]

Although mutation of Glu VI:23 had profound effects on norBNI and gNTI activity, it was postulated that other variant residues may help to confer selectivity through mechanisms of steric exclusion. One method of testing this hypothesis was by generating mutations that would give selective ligands enhanced affinity for their nonpreferred wild-type receptors. As a general example, if a bulky residue is blocking the access of a binding pocket, then replacement of this large residue with a smaller residue would significantly alter the binding affinity. On the other hand, small residues that allow numerous ligands access could be mutated to bulky residues so as to limit the number of ligands. One position that was hypothesized to play such a role was that of VII:03. When this position in κ was mutated, Tyr VII:03 to alanine (Ala), small changes in norBNI and gNTI binding were observed.[49] This suggested that the tyrosine was not directly involved in ligand binding. However, when the homologous residue in μ was mutated, Trp VII:03 to Ala, a significant increase in binding was observed.[49] Thus, it was suggested that the tryptophan in μ sterically excludes opiates with large address moieties from binding. The proposed pharmacophore for gNTI is presented in Figure 7 (the conserved aromatic residues from Figure 5 have been excluded for clarity).

Delta-Selective Opiates

The molecular recognition of the prototypical δ-selective opiates NTI and 7-spiroindanyloxymorphone[50] (SIOM)

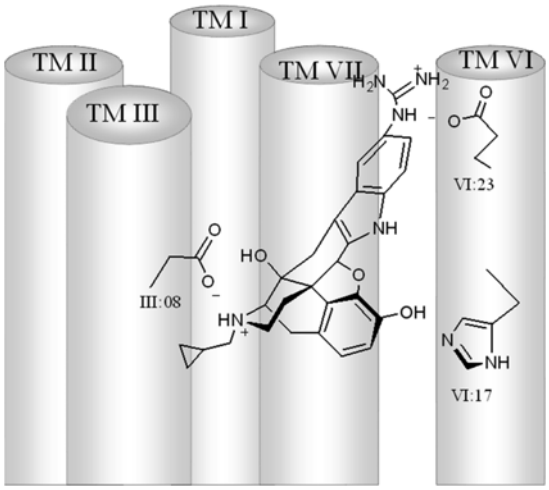

Figure 7. The pharmacophore of gNTI in the κ receptor. The "message" moiety forms a salt bridge with Asp III:08 and a hydrogen bond with His VI:17. The positively charged guanidinyl group is thought to form a salt bridge with Glu VI:23. This interaction is the basis for selectivity. Asp indicates aspartate; His, histidine; Glu, glutamine; TM, transmembrane.

(Figure 8) has also been well studied. Like their κ counterparts, δ-selective opiates have a distinctive "address" moiety. For these δ-selective ligands, however, a hydrophobic group such as an indole or spiroindane forms the address (NTI and SIOM, respectively). As mentioned previously, an alanine scan was conducted to determine whether residues within the address locus could be implicated in ligand binding. For naltrindole, the mutations that had the most pronounced effects were Trp VI:23, Leu VII:-04, and Ala VII:-01 (to glycene [Gly]).[47] Although no specific interaction with NTI was proposed, it is generally believed that these residues form a hydrophobic pocket that helps to stabilize the indolic moiety of NTI.

Similar to κ-selective opiates, δ-selective opiates appear to obtain their selectivity through mechanisms of exclusion. Again, site-directed mutagenesis studies were conducted at position VII:03. In the μ receptor, mutation of Trp VII:03 to Ala, Lys, or Leu (the residue found in wildtype δ) led to significantly increased binding affinities for NTI and SIOM.[49,51] This suggested that tryptophan blocks access of δ-selective opiates to the μ binding cavity. Also important to note is that the activity of NTI was similar for all 3 mutants, suggesting that the leucine is not directly involved in ligand binding.

Collectively, the above studies suggest that δ-selective opiates obtain selectivity through 2 main mechanisms. The first involves hydrophobic stabilization by residues unique to the δ receptor found at the top of TM VI and VII (Trp VI:23, Leu VII:-04, Ala VII:-01). The second mechanism implicates a tryptophan residue in μ that creates too much steric bulk. A depiction of these interactions appears in Figure 9.

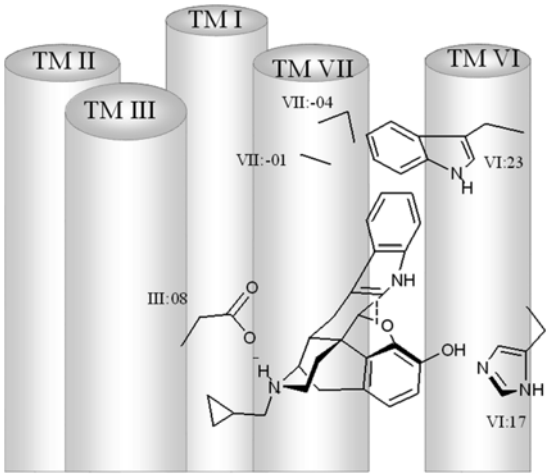

Figure 9. The pharmacophore of NTI in the δ receptor. Again, Asp III:08 and VI:17 anchor the tyramine moiety. Residues unique to the δ receptor at the top of TM VI and VII (Trp VI:23, Leu VII:-04, Ala VII:-01) confer selectivity. Presumably these residues form a hydrophobic pocket around the indolic moiety of NTI. Ala indicates alanine; Asp, aspartate; Leu, leucine; NTI, naltrindole; TM, transmembrane; Trp, tryptophan.

Mu-Selective Opiates

In the previous sections on κ and δ selectivity, it was apparent that one particular chemical moiety, the address, conferred selectivity. The μ-selective opiates morphine and β-funaltrexamine (β-FNA) (Figure 10) lack a common chemical moiety; thus, it is unlikely that common mechanisms exist for their molecular recognition. For this reason, they will be examined independently so that their unique structural features and their role in conferring selectivity can be discussed.

Morphine, the prototypical μ opiate, has been studied extensively. X-ray studies have determined that the C ring adopts a boat conformation, placing the 6α-hydroxyl group in an equatorial position.[13] It is often suggested that this hydroxyl group plays a role in selectivity. However, inversion of the hydroxyl group to the 6β diastereomer leads to only minor changes in selectivity.[13] Thus, it remains unclear how the hydroxyl group confers selectivity. One docking study suggests that it hydrogen-bonds to Asn V:02, a unique residue of the μ receptor.[30] Mutation of this residue to a leucine or a threonine moderately increases morphine's activity, thus casting serious doubts about this asparagine's putative role.[52] Another study used S-activated dihydromorphine derivatives, in combination with molecular mechanics, to create a pharmacophore. This model suggests that the 6α-hydroxyl group interacts with residues at the top of TM VII (near cysteine [Cys] VII:06), while the phenolic ring interacts with Tyr VI:19.[53] Although this model is plausible, it also lacks compelling site-directed mutagenesis data to support its claims, thereby diminishing its reliability.

The affinity label β-FNA is an irreversible μ antagonist. Although K_i values in the guinea pig brain show only slight

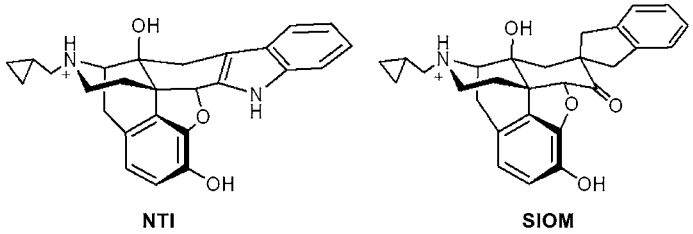

Figure 8. Delta-selective opiates NTI and SIOM. NTI indicates naltrindole; SIOM, 7-spiroindanyloxymorphone.

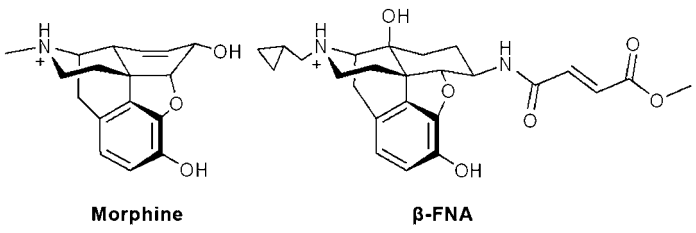

Figure 10. Mu-selective opiates morphine and β-FNA. β-FNA indicates β-funaltrexamine.

selectivity for μ over κ, it has been shown that at low concentrations [^{3}H] β-FNA covalently labels the μ receptor with high specificity.[54]Kinetic studies have suggested that the molecular recognition of this irreversible antagonist involves 2 steps.[54] First, reversible binding occurs within the binding pocket. This positions the reactive affinity label in the proper orientation to irreversibly react with a nucleophilic site within the receptor. Site-directed mutagenesis results have indicated that Lys V:05 is responsible for this attack.[55] Surprisingly, a lysine present at the homologous position of the κ receptor does not form a covalent bond with β-FNA. The molecular basis behind this puzzling result remains undetermined but presumably involves residues near Lys V:05 that alter the local environment of the κ binding pocket. β-FNA's interactions are highlighted in the pharmacophore in Figure 11.

Selective Nonopiate Ligands

In contrast to the opiates, there have not been any small-molecule ligands that show a high affinity for all 3 receptor types. Therefore, the remainder of this review will focus on the molecular recognition of the prototypical small-ligand agonists for each receptor type: the δ-selective diarylpiperazines (SNC 80[56] and analogs), the μ-selective fentanyls, and the κ-selective arylacetamides. Although exhaustive structure-activity relationships (SAR) data has determined which structural features are necessary for activity, the interactions that confer their selectivity are not well understood. In fact, conflicting pharmacophores have been proposed for each ligand class.[24-30] For the δ-selective SNC 80 derivatives, controversy exists over whether the pharmacophoric determinants of SNC 80 (and analogs) are the same as those for the δ-selective opiates NTI and SIOM.[57-59] For the fentanyls and arylacetamides, different yet important discrepancies exist.

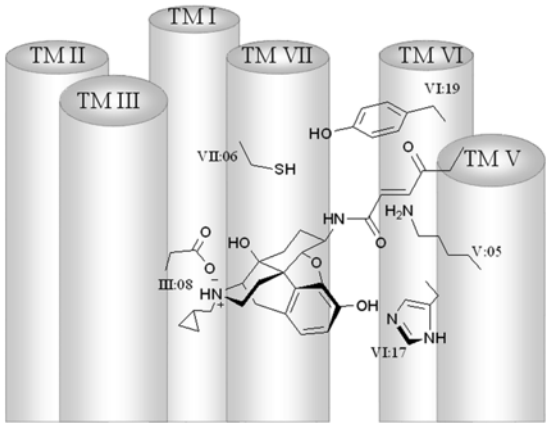

Figure 11. The pharmacophore of β-FNA in the μ receptor. Besides the typical "message" interactions, little is known about the residues that confer selectivity to this ligand. It has been suggested that Lys V:05 attacks the affinity label. Other residues in this putative pocket include those that were deemed important for morphine's recognition (Tyr VI:19 and Cys VII:06). β-FNA indicates β-funaltrexamine; Cys, cysteine; Lys, lysine; TM, transmembrane; Tyr, tyrosine.

Much of the controversy observed for these 3 ligand classes stems from the interpretation of automated docking studies. Their inherent flexibility creates numerous issues. Whereas the opiates produce a limited number of binding conformations, these flexible ligands often produce large ensembles of possible conformations within the receptor. This often leads to discrepancies in binding modes. Another issue that sometimes leads to inconsistent pharmacophores is the interpretation of site-directed mutagenesis. It is generally accepted that at least a 5-fold change in activity is needed before one can imply significance. This may or may not be true for such flexible ligands. Since these ligands have rotatable groups that are able to reposition themselves, the elimination of a single hydrophobic interaction may not give a "significant" difference in binding. This may lead to the elimination of pharmacophores that are presumed to not fit the experimental data. Collectively, all of these factors make the search for an "address" locus much more difficult. The scope of this review prevents an in-depth comparison of all details presented by each proposed model. Instead, key results from site-directed mutagenesis studies and chimeric studies will be highlighted to present a generalized pharmacophore.

SNC 80 and Analogs

The δ-selective agonist SNC 80 and its analogs (eg, compounds **1**[60] and **2**[61]) share several common structural features (Figure 12). One common feature is the presence of a piperidine or piperazine ring (ring A). Although substitution of this ring is not necessary, the addition of small hydrophobic groups sometimes leads to slightly enhanced binding affinity or selectivity.[62] Also common to these ligands are 2 aromatic rings (B and C). Ring B is generally substituted with a diethyl amide and ring C with a hydroxyl or methoxyether group. For a complete overview on SAR, the reader is referred to reviews by Calderon[63] and Knapp.[62]

Inspection of these ligands has suggested that they have structural similarities to δ-selective opiates NTI and SIOM.[64] A side-by-side comparison between **1** and SIOM reveals that rings A and C may mimic the classical opiate "message." Similarly, ring B and the diethyl amide mimic the spirocyclic

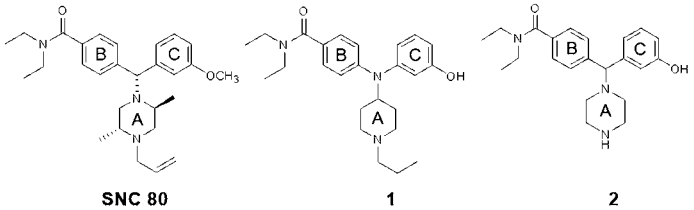

Figure 12. SNC 80 and its δ-selective analogs.

address group of SIOM (Figure 13). Accordingly, it was suggested that the ligands may be binding in a similar orientation, using the same residues that were found to impart δ-selectivity for the opiates. Based on this hypothesis, Dondio et al designed the SNC 80-indolomorphan mimic SB-220718.[65]

Despite Dondio's successful application of the proposed hypothesis, several studies have questioned the validity of such a simplistic model.[65-67] One area of disagreement lies in the analysis of SAR data. It is argued that since the diethyl amide in SB-220718 can be modified to an ester or a thioamide without a significant decrease in activity,[66] similar results should be seen for SNC 80 analogs with the same substitutions. However, this is not the case.[61] Other SAR inconsistencies are believed to occur at the 3′ position. In the opiates, the presence of a hydroxyl group is essential for activity,[13] but for SNC 80 it is not. Important to note is that this discrepancy is not consistent for all SNC 80 analogs. For example, the methoxy analog of **1** is nearly 100-fold less active than the hydroxyl analog.[62] Other analogs also show selectivity profiles in which the presence of a hydroxyl group is much greater.[65] This suggests that variations between structurally similar molecules (SNC 80 and **1**) can result in quite different SAR data.

Site-directed mutagenesis results also give clues that suggest whether these SNC 80 analogs share pharmacophoric overlap with the opiates.[55] As mentioned previously, a collection of residues near the putative δ address locus were randomly mutated to alanines. Binding results from this study revealed that Trp VI:23, Leu VII:-04, and Ala VII:-01 were important for NTI binding. The same study also reported that Trp VI:23, valine (Val) VII:-02, and Val VII:-03 were important for SNC 80 binding. Although Val VII:-02 and Val VII:-03 are not part of the 3 residues that were deemed to be important for NTI binding, they are located next to the ones that have been implicated. Therefore, it is reasonable to suggest that SNC 80 and its analogs share the same binding pocket as NTI but are oriented slightly differently (Figure 14).

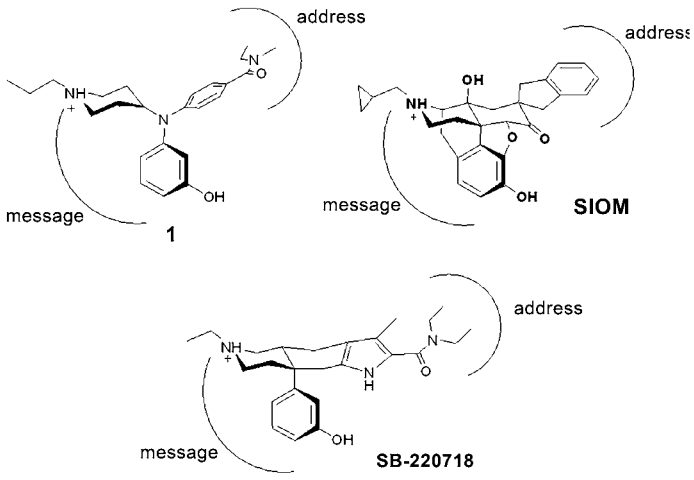

Figure 13. Message-address concept applied to δ-selective ligands lacking the traditional opiate core. SIOM indicates 7-spiroindanyloxymorphone.

Fentanyl and Analogs

Fentanyl and its congeners (the fentanyls) (Figure 15) are well known for their tremendous analgesic properties. Key to their pharmacological activity (μ-selective agonism) is the N-phenyl-N-piperidinyl propionamide moiety. Another group that is required for activity is a phenethyl group (although the phenyl ring can be substituted with a thiophene or methyl ester, as in the case of sufentanil and remifentanil). Other modifications that modulate activity include small moieties at the 3 or 4 position of the piperidine ring and a β-hydroxyl substitution. For an extensive overview on the SAR associated with these ligands, the reader is referred to a review by Casy et al.[67]

Despite exhaustive SAR data, the molecular recognition of the fentanyls remains largely unknown. Although it is accepted that the aminergic nitrogen interacts with Asp III:08, the nature of the interactions that confer fentanyl's selectivity is highly disputed. Mutational data from Lys VI:23 and Trp VII:03 (positions that were important for selective opiate recognition) suggest that these 2 residues are not involved in fentanyl's binding.

Docking methods have been used to suggest an alternative binding pocket. Unfortunately, fentanyl's flexibility makes such methods very difficult to interpret.[24-26] Potential bioactive conformations have been analyzed, yet the findings have not been conclusive.[30,68-70] In particular, the conformation of the phenethyl group has been the topic of numerous

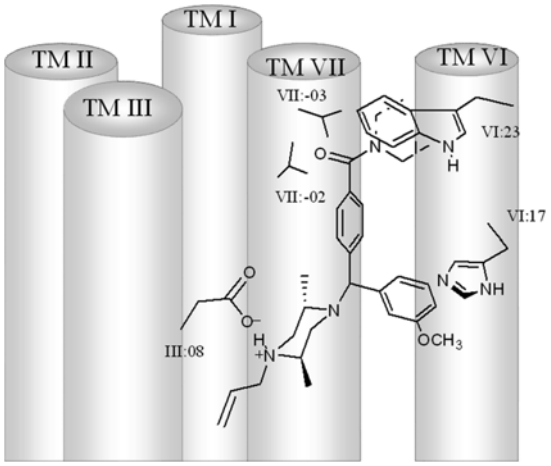

Figure 14. The pharmacophore of SNC 80 in the δ receptor. A selectivity locus is near the top of TM VI and VII. Presumably Trp VI:23, Val VII:-02, and Val VII:-03 form a hydrophobic pocket that stabilizes the diethylamide moiety. His VI:17 may participate in hydrophobic stacking or may hydrogen-bond when applicable. Additional selectivity may be observed depending on which interaction occurs.

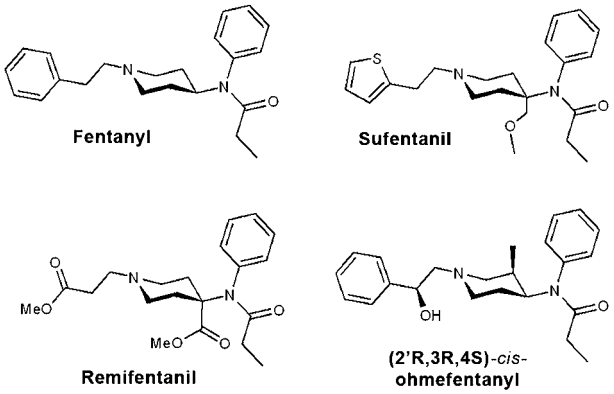

Figure 15. Fentanyl and its μ-selective analogs.

discussions. Although it has been predicted that the fentanyls prefer extended conformation, it is unclear whether the extended conformation is the bioactive form. One docking study suggests that the β-hydroxy group of the fentanyl interacts with Tyr III:09.[71] However, when this residue was mutated to phenylalanine, only a minor difference in binding was observed.[72] Another study suggests that the phenethyl group points up toward Lys III:02.[30] A third study suggests that the phenethyl moiety is projected deep within the cavity of TM II, III, and VII.[24] This final pharmacophoric model is the only one that maintains the phenethyl moiety in an extended conformation. Therefore, it will be the focus of the highlighted pharmacophore.

A notable feature of this pharmacophore (Figure 16) is the addition of an alternative address locus located deep within the cavity between TM II, III, and VII. This locus is supported by the increased μ activity of several compounds containing large aromatic moieties (generally phenethyl) positioned on the piperidine ring.[24,72] For example, the δ-selective ligand **1** shows enhanced activity for the μ receptor when a phenethyl moiety replaces the propyl-containing lead.[72] Additionally, it has been shown that N-phenethylmorphine shows greater potency than its parent compound morphine.[73] Also important to note is that this pocket contains several residues that can hydrogen-bond with the hydroxyl group of ohmefentanyl, specifically a threonine in TM II. However, since this residue is conserved, it is not apparent how it might contribute to ohmefentanyl's increased selectivity. Undoubtedly, more site-directed mutagenesis studies need to be conducted to help validate this pharmacophore.

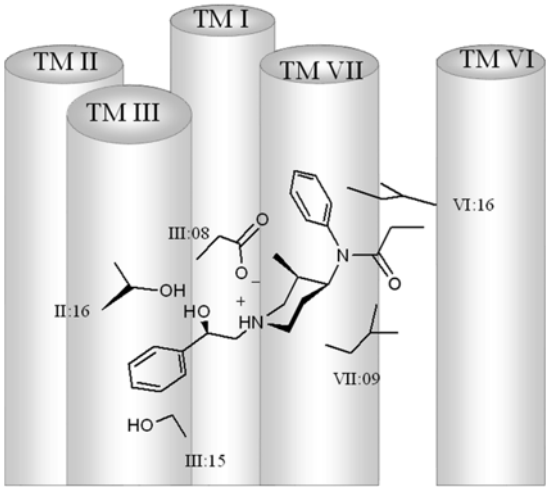

Figure 16. The pharmacophore of (2'R,3R,4S)-cis-ohmefentanyl in the μ receptor. The piperidine forms a salt bridge with Asp II:08. A putative hydrogen bonding interaction is thought to occur for Thr II:16. Other important interactions include Ile VI:16, Leu VII:09, and Ser III:15. Asp indicates aspartate; Ile, isoleucine; Leu, leucine; Ser, serine; Thr, threonine; TM, transmembrane.

Arylacetamides

The arylacetamides U50,488, U69,593, and CI-977[74] are potent κ-selective agonists (Figure 17). Structure-activity relationships have elucidated the chemical features necessary for binding.[75] In summary, SAR reveals that the trans-configuration of the amine and the amide moiety is essential. Compounds with altered stereochemistry at this position do not retain activity. The location of the amide moiety with respect to the aromatic moiety is also key. Reversing the amide or shortening the phenacetyl derivative to a benzamide derivative changes the binding activity significantly. SAR data also indicate that the pyrrolidine ring is optimal for both high affinity and selectivity.

Comparison of the arylacetamides to the κ-selective opiates does not lead to obvious similarities. Despite this fact, numerous attempts have been made to compare the 2.[76,77] Presumably, if a link between these 2 ligand classes could be established, it would be easier to model and design new κ-selective ligands. A study by Rajagopalan et al prepared numerous tetrahydronaphthalenes-U50,488 mimics.[78] This study observed that hydroxyl substitution on the 6' position led to compounds that achieved high affinity to both κ and μ receptors. When the 6' position was substituted with a methoxy ether (DuP-747), selectivity for the κ receptor was obtained, with little effect on the activity.[78]These tetrahydronapthalene-U50,488 mimics have since been used in molecular modeling studies[28] and compared with ethylketocyclazocine, a benzomorphan with slight κ-selectivity (Figure 18). Although it has been suggested that the molecular basis

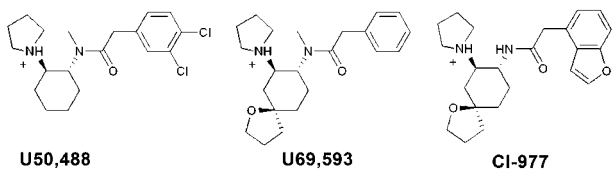

Figure 17. The κ-selective arylacetamides U50,488, U69,593, and CI-977.

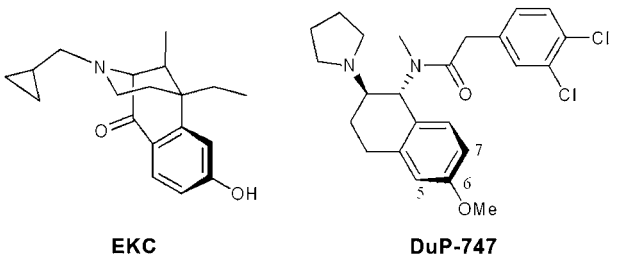

Figure 18. EKC and DuP 747, a κ-selective ligand structurally similar to the arylacetamides and benzomorphans. EKC indicates ethylketocyclazocine.

behind the increased κ activity is attributable to the interaction of the phenolic moiety with His VI:17, no attempts were made at determining the interactions responsible for cross-selectivity.[28]

Also important to note about the arylacetamides is the lack of a second cationic moiety. Thus, it was not unexpected when a mutation introduced at the traditional κ address site, Glu VI:23, led to only minimal effects on arylacetamide binding, suggesting no interaction.[79] However, other mutations in the traditional address locus did reveal significant changes in binding. For example, mutation of Tyr VII:03 to Ala led to significantly lower binding affinities.[79] Another mutation in this region that has shown importance is Ile VI:20. Mutation of this residue to lysine resulted in a decrease of CI-977's affinity by over 100-fold. Collectively, these results point toward a novel arylacetamide binding epitope that has the amine interacting with Asp III:08 and has residues Ile VI:20 and Tyr VII:03 playing distinct, albeit unknown roles (Figure 19). Binding models also suggest

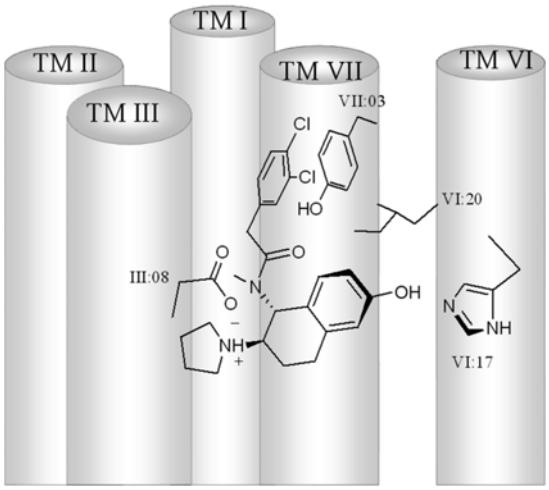

Figure 19. The pharmacophore of a DuP 747 analog in the κ receptor. It is suggested that Asp III:08 forms a salt bridge with the pyrrolidine ring. The phenolic ring is thought to interact with His VI:17. Other residues that have been implicated in binding (via site-directed mutagenesis) include Ile VI:20 and Tyr VII:03. that Leu VI:21 and Ala VI 24 also help to stabilize the arylacetamides.[28] With the exception of Asp III:08, all of these residues are unique to the κ receptor.

Conclusions

Pharmacophores have been presented for the prototypical opiate and nonopiate ligands (excluding peptides). For each ligands class, an attempt was made to present the model that, as far as we know, most accurately reflects the most current experimental data. However, since the interpretation of docking studies and site-directed mutation results often is subjective, there can be some debate as to whether the proposed model accurately depicts the true interactions. In some cases, such as that of gNTI, the experiments paint a clear picture of structure and function. However, for the majority of ligands, it is fairly clear that multiple sites of recognition exist. Comparisons between arylacetamides and SNC80 analogs, for example, indicate that these compounds most likely recognize different sites of selectivity within the opioid receptors. It is hoped that in the future, these ligand-specific sites will be identified and applied to design the next generation of opioid ligands.

References

1. Haeyoung K, Raynor K, Reisine T. Amino acids in the cloned mouse kappa receptor that are necessary for high affinity agonist binding but not antagonist binding. *Regul Pept*. 1994;54:155-156.

2. Meng F, Hoversten MT, Thompson RC, Taylor L, Watson SJ, Akil H. A chimeric study of the molecular basis of affinity and selectivity of the κ and the δ opioid receptors: potential role of extracellular domains. *J Biol Chem*. 1995;270:12730-12736.

3. Xue JC, Chen C, Zhu J, et al. The third extracellular loop of the μ opioid receptor is important for agonist selectivity. *J Biol Chem*. 1995;270:12977-12979.

4. Meng F, Ueda Y, Hoversten MT, et al. Mapping the receptor domains critical for the binding selectivity of delta-opioid receptor ligands. *Eur J Pharmacol*. 1996;311:285-292.

5. Wang JB, Johnson PS, Wu JM, Wang WF, Uhl GR. Human κ opiate receptor second extracellular loop elevates dynorphin's affinity for human μ/κ chimeras. *J Biol Chem*. 1994;269:25966-25969.

6. Teschemacher H, Opheim KE, Cox BM, Goldstein A. Peptidelike substance from pituitary that acts like morphine, I: isolation. *Life Sci*. 1975;16:1771-1775.

7. Strader CD, Sigal IS, Dixon RA. Structural basis of β-adrenergic receptor function. *FASEB J*. 1989;3:1825-1832.

8. Hibert MF, Trumpp-Kallmeyer S, Bruinvels A, Hoflack J. Three-dimensional models of neurotransmitter G-binding protein-coupled receptors. *Mol Pharmacol*. 1991;40:8-15.

9. Trumpp-Kallmeyer S, Hoflack J, Bruinvels A, Hibert M. Modeling of G-protein-coupled receptors: application to dopamine, adrenaline, serotonin, acetylcholine, and mammalian opsin receptors. *J Med Chem*. 1992;35:3448-3462.

10. Baldwin JM. The probable arrangement of the helices in G protein-coupled receptors. *EMBO J*. 1993;12:1693-1703.

11. Strader CD, Sigal IS, Candelore MR, Rands E, Hill WS, Dixon RAF. Conserved aspartic acid residues 79 and 113 of the β-adrenergic receptor have different roles in receptor function. *J Biol Chem*. 1988;263:10267-10271.

12. Strader CD, Candelore MR, Hill WS, Sigal IS, Dixon RA. Identification of two serine residues involved in agonist activation of the β-adrenergic receptor. *J Biol Chem*. 1989;264:13572-13578.

13. Lenz GR, Evans SM, Walters DE, Hopfinger AJ. *Opiates.* Orlando, FL: Academic Press; 1986.

14. Surratt CK, Johnson PS, Moriwaki A, et al. Mu opiate receptor. Charged transmembrane domain amino acids are critical for agonist recognition and intrinsic activity. *J Biol Chem*. 1994;269: 20548-20553.

15. Spivak CE, Beglan CL, Seidleck BK, et al. Naloxone activation of μ-opioid receptors mutated at a histidine residue lining the opioid binding cavity. *Mol Pharmacol*. 1997;52:983-992.

16. Schwyzer R, Eberle A. On the molecular mechanism of α-MSH receptor interactions. *Front Horm Res*. 1977;4:18-25.

17. Portoghese PS, Sultana M, Takemori AE. Design of peptidomimetic δ opioid receptor antagonists using the message-address concept. *J Med Chem*. 1990;33:1714-1720.

18. Resnick RB, Volavka J, Freedman AM, Thomas M. Studies of EN-1639A (naltrexone): a new narcotic antagonist. *Am J Psychiatry*. 1974;131:646-650.

19. Portoghese PS, Sultana M, Takemori AE. Naltrindole: a highly selective and potent non-peptide delta opioid receptor antagonist. *Eur J Pharmacol*. 1988;146:185-186.

20. Jones RM, Hjorth SA, Schwartz TW, Portoghese PS. Mutational evidence for a common κ antagonist binding pocket in the wild-type κ and mutant μ[K303E] opioid receptors. *J Med Chem*. 1998;41:4911-4914.

21. Magnan J, Paterson SJ, Tavani A, Kosterlitz HW. The binding spectrum of narcotic analgesic drugs with different agonist and antagonist properties. *Naunyn Schmiedebergs Arch Pharmacol*. 1982;319:197-205.

22. Szmuszkovicz J, Von Voightlander PF. Benzeneacetamide amines: structurally novel non-μ-opioids. *J Med Chem*. 1982;25: 1125-1126.

23. Lahti RA, Mickelson MM, McCall JM, Von Voigtlander PF. [^{3}H]U-69593 a highly selective ligand for the opioid kappa receptor. *Eur J Pharmacol*. 1985;109:281-284.

24. Subramanian G, Paterlini MG, Portoghese PS, Ferguson DM. Molecular docking reveals a novel binding site model for fentanyl at the μ-opioid receptor. *J Med Chem*. 2000;43:381-391.

25. Xu H, Lu YF, Partilla JS, et al. Opioid peptide receptor studies, 11: involvement of Tyr149, Trp318 and His319 of the rat μ-opioid receptor in binding of μ-selective ligands. *Synapse*. 1999;32:23-28.

26. Jiang HL, Huang XQ, Rong SB, et al. Theoretical studies on opioid receptors and ligands, I: molecular modeling and QSAR studies on the interaction mechanism of fentanyl analogs binding to μ-opioid receptor. *Int J Quantum Chem*. 2000;78:285-293.

27. Subramanian G, Paterlini MG, Larson DL, Portoghese PS, Ferguson DM. Conformational analysis and automated receptor docking of selective arylacetamide-based κ-opioid agonists. *J Med Chem*. 1998;41:4777-4789.

28. Lavecchia A, Greco G, Novellino E, Vittorio F, Ronsisvalle G. Modeling of κ-opioid receptor/agonists interactions using pharmacophore-based and docking simulations. *J Med Chem*. 2000;43:2124-2134.

29. Cappelli A, Anzini M, Vomero S, et al. Synthesis, biological evaluation, and quantitative receptor docking simulations of 2-[(acylamino)ethyl]-1,4-benzodiazepines as novel tifluadom-like ligands with high affinity and selectivity for κ-opioid receptors. *J Med Chem*. 1996;39:860-872.

30. Pogozheva ID, Lomize AL, Mosberg HI. Opioid receptor three-dimensional structures from distance geometry calculations with hydrogen bonding constraints. *Biophys J*. 1998;75:612-634.

31. Knapp RJ, Malatynska E, Collins N, et al. Molecular biology and pharmacology of cloned opioid receptors. *FASEB J*. 1995;9:516-525.

32. Jiang Q, Takemori AE, Sultana M, et al. Differential antagonism of opioid delta antinociception by [D-Ala2,Leu5,Cys6]enkephalin and naltrindole 5′-isothiocyanate: evidence for delta receptor subtypes. *J Pharmacol Exp Ther*. 1991;257:1069-1075.

33. Sofuoglu M, Portoghese PS, Takemori AE. 7-Benzylidenenaltrexone (BTNX): a selective Δ_1 opioid receptor antagonist in the mouse spinal cord. *Life Sci*. 1993;52:769-775.

34. Zaki PA, Bilsky EJ, Vanderah TW, Lai J, Evans CJ, Porreca F. Opioid receptor types and subtypes: the δ receptor as a model. *Annu Rev Pharmacol Toxicol*. 1996;36:379-401.

35. Stenkamp RE, Filipek S, Driessen CA, Teller DC, Palczewski K. Crystal structure of rhodopsin: a template for cone visual pigments and other G protein-coupled receptors. *Biochim Biophys Acta*. 2002;1565:168-182.

36. Filipek S, Teller DC, Palczewski K, Stenkamp R. The crystallographic model of rhodopsin and its use in studies of other G protein-coupled receptors. *Annu Rev Biophys Biomol Struct*. 2003;32:375-397.

37. Metzger TG, Paterlini MG, Portoghese PS, Ferguson DM. Application of the message-address concept of the docking of naltrexone and selective naltrexone-derived opioid antagonists into opioid receptor models. *Neurochem Res*. 1996;21:1287-1294.

38. Alkorta I, Loew GH. A 3D model of the δ opioid receptor and ligand-receptor complexes. *Protein Eng*. 1996;9:573-583.

39. Strahs D, Weinstein H. Comparative modeling and molecular dynamics studies of the δ, κ and μ opioid receptors. *Protein Eng*. 1997;10:1019-1038.

40. Meng EC, Shoichet BK, Kuntz ID. Automated docking with grid-based energy evaluation. *J Comput Chem*. 1992;13:505-524.

41. Blumberg H, Dayton HB, Wolf PS. Counteraction of narcotic antagonist analgesics by the narcotic antagonist naloxone. *Proc Soc Exp Biol Med*. 1966;123:755-758.

42. Pasternak GW, Snyder SH. Opiate receptor binding: enzymic treatments that discriminate between agonist and antagonist interactions. *Mol Pharmacol*. 1975;11:478-484.

43. Beckett AH, Casy AF. Synthetic analgesics: stereochemical considerations. *J Pharm Pharmacol*. 1954;6:986-1001.

44. Attwood TK, Findlay JBC. Fingerprinting G-protein-coupled receptors. *Protein Eng*. 1994;7:195-203.

45. Befort K, Tabbara L, Kling D, Maigret B, Kieffer BL. Role of aromatic transmembrane residues on the δ-opioid receptor in ligand recognition. *J Biol Chem*. 1996;271:10161-10168.

46. Hjorth SA, Thirstrup K, Grandy DK, Schwartz TW. Analysis of selective binding epitopes for the κ-opioid receptor antagonist nor-binaltorphimine. *Mol Pharmacol*. 1995;47:1089-1094.

47. Valiquette M, Vu HK, Yue SY, Wahlestedt C, Walker P. Involvement of Trp-284, Val-296, and Val-297 of the human δ-opioid receptor in binding of δ-selective ligands. *J Biol Chem*. 1996;271: 18789-18796.

48. Portoghese AS, Lipkowski AW, Takemori AE. Bimorphinans as highly selective, potent κ opioid receptor antagonists. *J Med Chem*. 1987;30:238-239.

49. Metzger TG, Paterlini MG, Ferguson DM, Portoghese PS. Investigation of the selectivity of oxymorphone- and naltrexone-derived ligands via site-directed mutagenesis of opioid receptors: exploring the 'address' recognition locus. *J Med Chem*. 2001;44:857-862.

50. Portoghese PS, Moe ST, Takemori AE. A selective δ_1 opioid receptor agonist derived from oxymorphone. Evidence for separate recognition sites for δ_1 opioid receptor agonists and antagonists. *J Med Chem*. 1993;36:2572-2574.

51. Bonner G, Meng F, Akil H. Selectivity of μ-opioid receptor determined by interfacial residues near third extracellular loop. *Eur J Pharmacol*. 2000;403:37-44.

52. Pil J, Tytgat J. The role of the hydrophilic Asn230 residue of the μ-opioid receptor in the potency of various opioid agonists. *Br J Pharmacol*. 2001;134:496-506.

53. Kanematsu K, Sagara T. An approach to the rational design of opioid receptor ligands: non-narcotic κ-opioid receptor ligand KT-95 free from euphoria and/or dysphoria. *Curr Med Chem CNS Agents*. 2001;1:1-25.

54. Liu-Chen LY, Li SX, Tallarida RJ. Studies on kinetics of [^{3}H]β-funaltrexamine binding to μ opioid receptor. *Mol Pharmacol*. 1990;37:243-250.

55. Chen C, Yin J, Riel JK, et al. Determination of the amino acid residue involved in [^{3}H]β-funaltrexamine covalent binding in the cloned rat μ-opioid receptor. *J Biol Chem*. 1996;271:21422-21429.

56. Calderon SN, Rothman RB, Porreca F, et al. Probes for narcotic receptor mediated phenomena, 19: synthesis of (+)-4-[(αR)-α-((2S,5R)-4-allyl-2,5-dimethyl-1-piperazinyl)-3-methoxybenzyl]-N,N-diethylbenzamide (SNC 80): a highly selective, nonpeptide δ opioid receptor agonist. *J Med Chem*. 1994;37:2125-2128.

57. Liao S, Alfaro-Lopez J, Shenderovich MD, et al. De novo design, synthesis, and biological activities of high-affinity and selective non-peptide agonists of the delta-opioid receptor. *J Med Chem*. 1998;41:4767-4776.

58. Coop A, Jacobson AE. The LMC delta opioid recognition pharmacophore: comparison of SNC80 and oxymorphindole. *Bioorg Med Chem Lett*. 1999;9:357-362.

59. Bernard D, Coop A, MacKerell AD. 2D conformationally sampled pharmacophore: a ligand-based pharmacophore to differentiate delta opioid agonists from antagonists. *J Am Chem Soc*. 2003;125:3101-3107.

60. Carson JR, Carmosin FJ, Fitzpatrick LJ, Reitz AB, Jetter MC, inventors. 4-[aryl(piperidin-4-yl)]aminobenzamides.US patent 6 436 959. December 23, 1998.

61. Wei ZY, Brown W, Takasaki B, et al. N,N-Diethyl-4-(phenylpiperidin-4-ylidenemethyl)benzamide: a novel, exceptionally selective, potent δ opioid receptor agonist with oral bioavailability and its analogues. *J Med Chem*. 2000;43:3895-3905.

62. Knapp RJ, Santoro G, De Leon IA, et al. Structure-activity relationships for SNC80 and related compounds at cloned human delta and mu opioid receptors. *J Pharmacol Exp Ther*. 1996;277:1284-1291.

63. Calderon SN, Coop A. SNC 80 and related δ opioid agonists. *Curr Pharm Des*. 2004;10:733-742.

64. Podlogar BL, Poda GI, Demeter DA, et al. Synthesis and evaluation of 4-(N,N-diarylamino)piperidines with high selectivity to the δ-opioid receptor: a combined 3D-QSAR and ligand docking study. *Drug Des Discov*. 2000;17:34-50.

65. Dondio G, Ronzoni S, Eggleston DS, et al. Discovery of a novel class of substituted pyrrolooctahydroisoquinolines as potent and selective δ opioid agonists, based on an extension of the message-address concept. *J Med Chem*. 1997;40:3192-3198.

66. Dondio G, Ronzoni S, Petrillo P, Desjarlais RL, Raveglia LF. Pyrrolooctahydroisoquinolines as potent and selective δ opioid receptor ligands: SAR analysis and docking studies. *Bioorg Med Chem Lett*. 1997;7:2967-2972.

67. Casy AF, Parfitt RT. *Opioid Analgesics: Chemistry and Receptors.* New York, NY: Plenum Press; 1986.

68. Subramanian G, Ferguson DM. Conformational landscape of selective μ-opioid agonists in gas phase and in aqueous solution: the fentanyl series. *Drug Des Discov*. 2000;17:55-67.

69. Cometta-Morini C, Loew GH. Development of a conformational search strategy for flexible ligands: a study of the potent mu-selective opioid analgesic fentanyl. *J Comput Aided Mol Des*. 1991;5:335-356.

70. Brandt W, Barth A, Holtje HD. A new consistent model explaining structure (conformation)-activity relationships of opiates with μ-selectivity. *Drug Des Discov*. 1993;10:257-283.

71. Tang Y, Chen KX, Jiang HL, Wang ZX, Ji RY, Chi ZQ. Molecular modeling of μ opioid receptor and its interaction with ohmefentanyl. *Zhongguo Yao Li Xue Bao*. 1996;17:156-160.

72. Xu H, Lu YF, Partilla JS, et al. Opioid peptide receptor studies, 11: involvement of Tyr149, Trp318 and His319 of the rat μ-opioid receptor in binding of μ-selective ligands. *Synapse*. 1999;32:23-28.

73. Winter CA, Orahovats PD, Lehman EG. Analgesic activity and morphine antagonism of compounds related to nalorphine. *Arch Int Pharmacodyn Ther*. 1957;110:186-202.

74. Hunter JC, Leighton GE, Meecham KG, et al. CI-977, a novel and selective agonist for the κ-opioid receptor. *Br J Pharmacol*. 1990;101:183-189.

75. Rees DC. Chemical structures and biological activities of non-peptide selective kappa opioid ligands. *Prog Med Chem*. 1992;29:109-139.

76. Froimowitz M, DiMeglio CM, Makriyannis A. Conformational preferences of the κ-selective opioid agonist U50488. A combined molecular mechanics and nuclear magnetic resonance study. *J Med Chem*. 1992;35:3085-3094.

77. Higginbottom M, Nolan W, O'Toole J, Ratcliffe GS, Rees DC, Roberts E. The design and synthesis of κ opioid ligands based on a binding model for κ agonists. *Bioorg Med Chem Lett*. 1993;3:841-846.

78. Rajagopalan P, Scribner RM, Pennev P, et al. Dup 747: sar study. *Bioorg Med Chem Lett*. 1992;2:721-726.

79. Thirstrup K, Hjorth SA, Schwartz TW. Investigation of the binding pocket in the kappa opioid receptor by a combination of alanine substitutions and steric hindrance mutagenesis. Poster M30. 27th Meeting of the International Narcotics Research Conference; July 21-26, 1996; Long Beach, CA.