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Molecular recognition at kappa opioid receptors*

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Abstract: Structure–activity relationships are rarely straightforward, and often are more complicated than they appear. For this reason, the use of site-directed mutagenesis as a complementary tool to analyze structure–activity relationships has been invaluable. Here, we illustrate how site-directed mutagenesis has led to greater insight into the molecular basis for molecular recognition of norbinaltorphimine and to the design of novel kappa antagonists. Given the paucity of high-resolution crystal structures for membrane-bound receptors, the use of a coordinated "two-dimensional" paradigm that involves molecular modification of both the ligand and the receptor, affords a useful approach to the study of molecular recognition. This paradigm has led to the design of highly potent and selective kappa opioid receptor antagonists that are derivatives of the delta opioid receptor antagonist, naltrindole.

INTRODUCTION

The discovery of the endogenous opioid peptides, Leu- and Met-enkephalin, and their interaction with specific receptors was a turning point in opioid research [1]. These revelations confirmed the 1954 concept [2] of a specific "analgesic receptor" that recognizes morphine and highlighted the importance of an endogenous opioid system in the central nervous system. The endogenous opioid peptide family is characterized by a common tetrapeptide sequence (Tyr–Gly–Gly–Phe), and it now comprises a group of over a dozen ligands [3]. More recently, two additional endogenous ligands, endomorphin-1 and -2 (Tyr-Pro–X–Phe–NH_2 X = Trp or Phe), whose tetrapeptide sequence differs somewhat from that of the classical opioid peptides, have been reported [4].

Multiple opioid receptors and multiple modes of binding to opioid receptors were first proposed over three decades ago based on structure–activity relationship (SAR) analysis [5]. Subsequent *in vivo* and *in vitro* pharmacological studies led to the identification of three major types of opioid receptors, named delta, kappa, and mu [6,7]. With the cloning of these receptors in 1992–1993, a new chapter in opioid research was opened because their known amino acid sequences made it possible, for the first time, to investigate ligand–receptor interactions from the perspective of *both* the ligand and the receptor [8].

The opioid receptor family is a member of the rhodopsin subfamily in the superfamily of over 1000 G protein-coupled receptors. Members of the opioid receptor family are highly homologous (~60% amino acid identity) and recognize structurally diverse ligands that include peptides, opiates, and a variety of synthetic non-peptides [8]. The diversity of ligands, both endogenous and exogenous, and the multiplicity of opioid receptors provide a distinct advantage in exploring the basis for molecular recognition among G protein-coupled receptors in general, and opioid receptors in particular. This pres-

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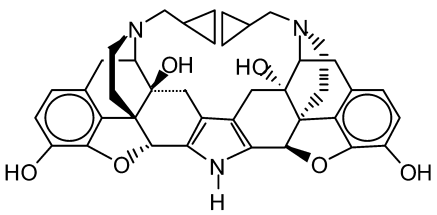
entation illustrates how site-directed mutagenesis and molecular modeling in conjunction with classical SAR studies proves to be a powerful combination for identifying the basis for molecular recognition of ligands at kappa opioid receptors.

MOLECULAR RECOGNITION OF NORBINALTORPHIMINE AT THE KAPPA OPIOID RECEPTOR

Norbinaltorphimine [9] **1** (norBNI) is the prototypical kappa opioid receptor antagonist, and it is employed widely as a tool in opioid research [10]. Structure–activity relationship studies in smooth muscle preparations have supported the important role of the N17′ basic nitrogen. Most noteworthy, was the finding that neutralization of N17′ through amidation greatly reduced the antagonist potency and selectivity at kappa receptors [11]. Also, an analog **2** without the critical groups required for an antagonist pharmacophore in the second half of the molecule, retained activity [12]. This suggested that the decahydroisoquinoline moiety within the second pharmacophore of norBNI acts as a scaffold to rigidly hold and direct its N17′ basic nitrogen to a subsite that is unique to the kappa receptor. Given that an analog of norBNI, with an isosteric thiophene [13] in place of the pyrrole moiety, possesses binding selectivity similar to that of norBNI, and the finding that a bivalent ligand whose scaffold geometry differs substantially from that of norBNI are not kappa-selective [14], supports the important directive role of the scaffold. Consequently, the binding of norBNI to the kappa receptor was postulated to involve two major subsites: The first subsite recognizes one of the antagonist pharmacophores, while the second subsite containing an anionic group associates with the cationic protonated N-17′ moiety in the second half of the molecule.



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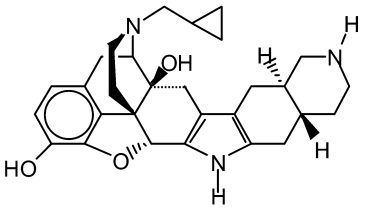

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This model bears a formal resemblance to the message–address concept proposed by Schwyzer [15], who employed it to analyze the structure–activity relationship of ACTH and related peptide hormones. Accordingly, peptide hormones contain a "message" sequence and "address" sequence. The message component is a common molecular feature of the series that is recognized by a family of receptors. The address recognizes a unique subsite on one of the receptors in the family and provides additional binding affinity to the ligand.

Site-directed mutagenesis of the kappa receptor revealed the "address" subsite to be the anionic residue, Glu297, located at the top of the transmembrane spanning helix 6 (TM6) (Fig. 1) [16].

In this regard, the Glu297Lys and Glu297Ala mutants possessed greatly reduced affinity for norBNI , whereas the affinity of naltrexone was not affected. Homology modeling suggested that the residue corresponding to Glu297 is a tryptophan (Trp284) in the delta opioid receptor and a lysine (Lys303) in the mu opioid receptor. Given that the non-conserved Glu297 is primarily responsible for conferring high affinity to norBNI, residues at the equivalent position in the mu (Lys303) and delta (Trp284) receptors would tend to hinder binding through electrostatic repulsion in the former or steric hindrance in the latter. In the mu receptor, additional steric hindrance appears to arise from Trp318 located at the top of TM7, as we have determined that the Trp318Ala mutant has greatly increased affin-

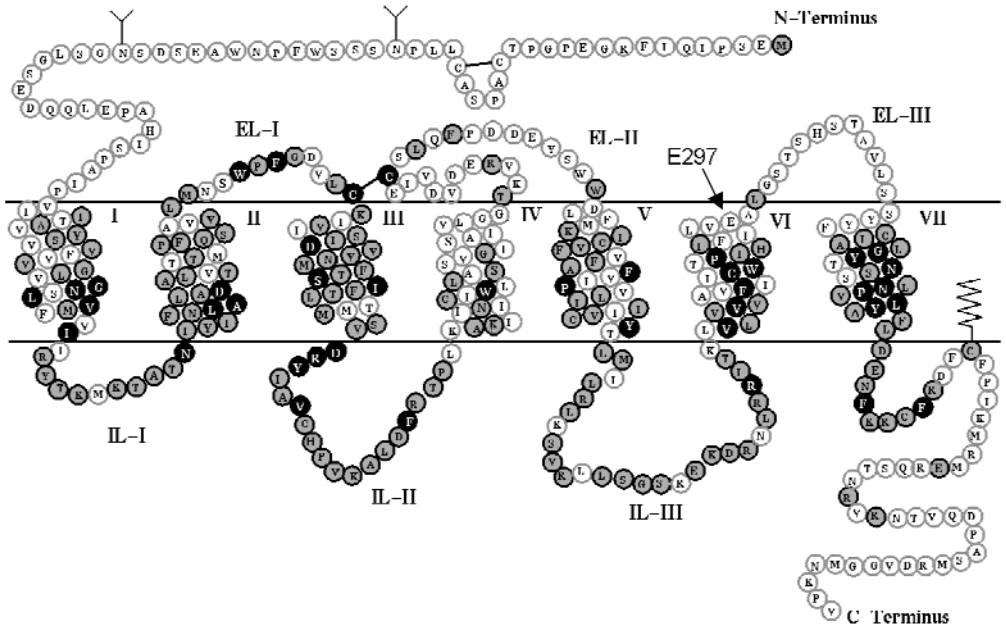


Fig. 1 A serpentine diagram of the κ receptor. Gray circles represent residues that are conserved among the three opioid receptors. Black circles represent residues identical with those in rhodopsin.

ity for norBNI [17]. The increased affinity most likely is due to the smaller size of Ala relative to Trp, thereby making the binding pocket more accessible to norBNI.

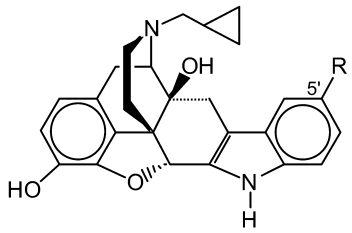
The role of Glu297 as an anionic residue that interacts with the cationic "address" of kappa antagonists has received added support from studies with the mu receptor mutant whose Lys303 was replaced by glutamate (Lys303Glu) [18]. The delta receptor was also mutated in an equivalent position (Trp284Glu) [17]. In these mutants, the glutamate residue is in a position equivalent to that of Glu297 in the kappa receptor. The mutant mu and delta receptors displayed enhanced affinity for norBNI and its active congeners when compared to the wild-type receptors, again highlighting the critical role of Glu297 in the kappa receptor. These data support the proposal [19] that the "message" recognition locus for the antagonist pharmacophore of norBNI and other naltrexone-derived ligands is the central cavity created by the 7TM helices, as this domain has the highest homology of the potential binding sites. A key conserved residue that interacts with the antagonist pharmacophore within this cavity is believed to be the conserved TM3 aspartate [20].

DESIGN OF NOVEL KAPPA OPIOID RECEPTOR ANTAGONISTS

In view of the structural requirements for the kappa antagonist activity of norBNI **1**, and the importance of a rigid scaffold for the orientation of its N17′ basic nitrogen, we have combined an indole scaffold with new "address" moieties in order to design novel kappa antagonists [21]. Superposition of the conserved structural motif of the delta-opioid receptor antagonist, naltrindole **3** (NTI) [22], upon that of norBNI, led to the use of the indole moiety as a rigid scaffold for projecting a variety of protonated amine or cationic groups within ion-pairing distance of the Glu297 residue in the kappa receptor.

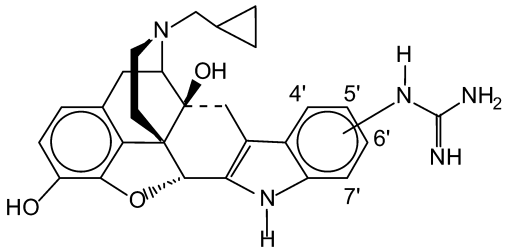
Ligands with high kappa antagonist potency contained 5′-substituted guanidinium, amidinium, amine, or quaternary ammonium substituents [21]. The high basicity of guanidines and amidines, and

the fact that the quaternary ammonium group is totally ionized, strongly support the notion that a cationic group at the 5′-position is required for potent kappa antagonist activity. The importance of a cationic 5′-substituent for kappa antagonist activity was shown by comparing the potency of the 5′-guanidine compound **4** (GNTI) with its closely related *N*-cyano derivative **5**. GNTI **3** was approximately 30-fold more potent than the nonbasic cyanoguanidine, highlighting the contribution of a positively charged group at the 5′-position of the indole scaffold. Binding studies of GNTI on wild-type and Glu297Lys mutant kappa receptors have implicated the involvement of counterionic association between the cationic 5′-guanidinium group and the carboxylate group of Glu297 [19].



3 R = H
4 R = NH(C=NH)NH_2
5 R = NH(C=N-CN)NH_2

The importance of the 5′-position for directing the basic group to the glutamate-containing locus on the receptor was demonstrated by the finding that the 4′-, 6′-, and 7′-regioisomers (**6a–c**) either were inactive as antagonists or had greatly reduced kappa antagonist potency [23]. Significantly, the 6′-regioisomer **6b** showed potent kappa agonist activity (~50-fold greater than morphine in the GPI) and a significant decrease in binding to the Glu297Ala mutant kappa receptor. These data have suggested that an ionic interaction between the 6′-guanidinium group and the Glu297 residue at the top of TM6 may be associated with the kappa agonist activity of **6b**. This unprecedented transition from potent antagonist (**4**) to potent agonist (**6b**) can be rationalized by a ligand-induced conformational change of TM6 in the kappa receptor. A change of the guanidinium group from the 5′- to 6′-position may result in a counterclockwise axial rotation of TM6 (as viewed extracellularly) in order for the counterions to maintain favorable association. Such axial motion would lead to a conformational change of inner-loop 3, which is the key domain involved in G protein coupling and activation. There are a number of reports that implicate the rotation of TM6 in this process [24–29].



Regioisomer	
4'	**6a**
6'	**6b**
7'	**6c**

REFERENCES

1. J. Hughes, T. N. Smith, H. W. Kosterlitz, L. A. Fothergill, B. A. Morgen, H. R. Morris. *Nature* **258**, 577–579 (1976).
2. A. H. Beckett and A. F. Casy. *J. Pharm. Pharmacol.* **6**, 986–1001 (1954).
3. E. J. Simon and J. M. Hiller. In *Basic Neurochemistry*, 5th ed., G. J. Siegel, B. W. Agranoff, R. W. Albers, P. B. Molinoff (Eds.), pp. 321–339, Raven Press, New York (1993).
4. J. E. Zadina, L. Hackler, L.-J. Ge, A. J. Kastin. *Nature* **386**, 499–502 (1997).
5. P. S. Portoghese. *J. Med. Chem.* **8**, 609–616 (1965).
6. W. R. Martin, C. G. Eades, J. A. Thompson, R. E. Huppler, P. E. Gilbert. *J. Pharmacol. Exp. Ther.* **197**, 517–532 (1976).
7. J. A. H. Lord, A. A. Waterfield, J. Hughes, H. W. Kosterlitz. *Nature* **267**, 495–499 (1977).
8. B. N. Dhawan, F. Cesselin, R. Raghubir, T. Reisine, P. B. Bradley, P. S. Portoghese, M. Hamon. *Pharmacol. Rev.* **48**, 567–592 (1996).
9. P. S. Portoghese, A. W. Lipkowski, A. E. Takemori. *J. Med. Chem.* **30**, 238–239 (1987).
10. A. E. Takemori and P. S. Portoghese. *Annu. Rev. Pharmacol. Toxicol.* **32**, 239–269 (1992).
11. P. S. Portoghese, C.-E. Lin, F. Farouz-Grant, A. E. Takemori. *J. Med. Chem.* **37**, 1495–1500 (1994).
12. C. E. Lin, A. E. Takemori, P. S. Portoghese. *J. Med. Chem.* **36**, 2412–2415 (1993).
13. P. S. Portoghese, A. Garzon-Aburbeh, H. Nagase, C. E. Lin, A. E. Takemori. *J. Med. Chem.* **34**, 1292–1296 (1991).
14. M. L. Bolognesi, W. H. Ojala, W. B. Gleason, J. F. Griffin, F. Farouz-Grant, D. L. Larson, A. E. Takemori, P. S. Portoghese. *J. Med. Chem.* **39**, 1816–1822 (1996).
15. R. Schwyzer. *Ann. N.Y. Acad. Sci.* **247**, 3–26 (1997).
16. S. A. Hjorth, K. Thirstrup, D. K. Grandy, T. W. Schwartz. *Mol. Pharmacol.* **47**, 1089-1094 (1995).
17. T. G. Metzger, M. G. Paterlini, D. M. Ferguson, P. S. Portoghese. *J. Med. Chem.* **43**, 857–862 (2001).
18. D. L. Larson, R. M. Jones, S. A. Hjorth, T. W. Schwartz, P. S. Portoghese. *J. Med. Chem.* **43**, 1573–1576 (2000).
19. R. M. Jones, S. A. Hjorth, T. W. Schwartz, P. S. Portoghese. *J. Med. Chem.* **41**, 4911–4914 (1998).
20. T. G. Metzger, M. G. Paterlini, P. S. Portoghese, D. M. Ferguson. *Neurochem. Res.* **21**, 1287–1294 (1996).
21. W. C. Stevens, R. M. Jones, G. Subramanian, T. G. Metzger, D. M. Ferguson, P. S. Portoghese. *J. Med. Chem.* **43**, 2759–2769 (2000).
22. P. S. Portoghese, M. Sultana, A. E. Takemori. *J. Med .Chem.* **33**, 1714–1720 (1990).
23. S. K. Sharma, R. Jones, T. G. Metzger, D. M. Ferguson, P. S. Portoghese. *J. Med. Chem.* **44**, 2072–2079 (2001).
24. D. L. Farrens, C. Altenbach, K. Yang, W. L. Hubbell, H. G. Khorana. *Science* **274**, 768–770 (1996).
25. U. Gether, S. Lin, P. Ghanouni, J. A. Ballesteros, H. Weinstein, B. K. Kobilka. *Embo. J.* **16**, 6737–6747 (1997).
26. J. A. Javitch, D. Fu, G. Liapakis, J. Chen. *J. Biol. Chem.* **272**, 18546–18549 (1997).
27. S. G. F. Rasmussen, A. D. Jensen, G. Liapakis, P. Ghanouni, J. A. Javitch, U. Gether. *Mol. Pharmacol.* **56**, 175–184 (1999).