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SELECTIVE NATREXONE-DERIVED OPIOID RECEPTOR ANTAGONISTS

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INTRODUCTION

Opioid antagonists, especially naloxone **1** and naltrexone **2,** have been widely employed as tools in opioid research (1, 2). The major criterion for the classification of an agonist effect as opioid-receptor-mediated is the ability of these antagonists to competitively antagonize the effect. The fact that they are universal opioid antagonists has made them useful for this purpose.

As it is now well established that there are multiple types of opioid receptors (3), there has been greater emphasis on the development and use of highly selective opioid antagonists (2). Two major advantages of selective antagonists are that they can be employed to evaluate the selectivity of new

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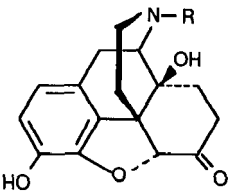


1 NALOXONE, R = CH_3

2 NALTREXONE, R = $CH_2CH(CH_2)_2$

agonists, and they are of value in probing the interaction of endogenous opioid peptides with opioid receptor types and subtypes.

The utility of antagonists as pharmacologic tools in many cases depends upon the reliable correlation of in vitro with in vivo activity. Nonpeptide ligands are preferred in such cases because they generally can penetrate the CNS and are less subject to metabolic inactivation. Nonpeptide selective opioid antagonists also have potential clinical applications in the treatment of a variety of disorders where endogenous opioids may play a modulatory role. These disorders include constipation, immune function, drug addiction, and alcoholism, to name only a few (4).

Naltrexone is superior to naloxone as an opioid antagonist because of its greater potency and bioavailability. This superiority has led to increased use of naltrexone, both as an experimental tool and clinically. For these reasons, many of the approaches to developing selective nonpeptide opioid antagonists have involved the chemical modification of naltrexone (5). Consequently, this review focuses on selective naltrexone-derived antagonists that interact non-covalently with opioid receptors.

RECEPTOR SELECTIVITY

Since this review discusses *selective* antagonists, the term "selective" deserves comment. A selective ligand has a greater pharmacologic effect or higher affinity associated with the target receptor relative to other receptors (6). In contrast, a *specific* ligand interacts with a single receptor population. Few, if any, ligands that act at opioid receptors appear to be specific.

Upon structural modification, the selectivity of an opioid antagonist can arise by basically three different mechanisms. This selectivity can occur when modification leads to (*a*) a large decrease in ligand affinity for nontarget sites, with smaller affinity changes for the target site; (*b*) a large increase in affinity

for the target site and smaller affinity changes at nontarget sites; or (c) large reciprocal changes for target and nontarget sites. Selectivity arising by mechanism (*a*) is exemplified by the δ antagonist, (Allyl$_2$Tyr-Aib-Aib-Phe-Leu (ICI174864), whose selectivity is due to very low affinity for non-δ sites (7). Its affinity for δ sites is similar to that of naltrexone, which is μ-selective. An example of selectivity that conforms to mechanism (*c*) has been reported with naltrindole (NTI)[1] (8). Its affinity is derived through greatly increased affinity for δ sites and decreased affinity for non-δ sites relative to its precursor, naltrexone.

Finally, it should be recognized that because opioid receptor types have not been cloned there are presently no "gold standards" for selective antagonists. More conclusive data concerning selectivity await verification of homogeneous opioid receptor types and subtypes.

DESIGN APPROACHES

The rationale for the design of naltrexone-derived κ- and δ-selective opioid antagonists has come from two different approaches that share the concept of simultaneous occupation of two neighboring recognition sites by a single ligand. This concept is embodied in the bivalent ligand and the message-address models (5).

Bivalent Ligand Approach and the Development of Selective κ Opioid Receptor Antagonists

The term bivalent ligand is defined as a molecule that contains two recognition units linked through a spacer (5). Bivalent ligands are classified into two groups; (*a*) those that contain two pharmacophores and (*b*) those that possess a single pharmacophore connected to a nonpharmacophore recognition unit.

The expectation of enhanced selectivity and potency of such ligands was based originally on a model in which the recognition units of a double pharmacophore bivalent ligand molecule "bridge" two neighboring opioid recognition sites (9). It was envisioned that each opioid receptor type may possess a unique organization that defines the distance between neighboring

[1]Abbreviations used: guinea pig ileum, GPI; norbinaltorphimine 8, norBNI; binaltorphimine 9, BNI; natrindole 15, NTI; benzofuran analogue 16, NTB; corticotropin-releasing factor, CRF; mouse vas deferns, MVD; [D-Ala^2,MePhe^4,Gly-ol^5]enkephalin, DAMGO; [D-Pen^2, D-Pen^5]enkephalin, DPDPE; [D-Ser^2,Leu^5]enkephalin-Thr, DSLET; luteinizing hormone, LH; N-methyl NTI, N-Me-NTI; β-funaltrexamine, β-FNA; ethylketazocine, EK.

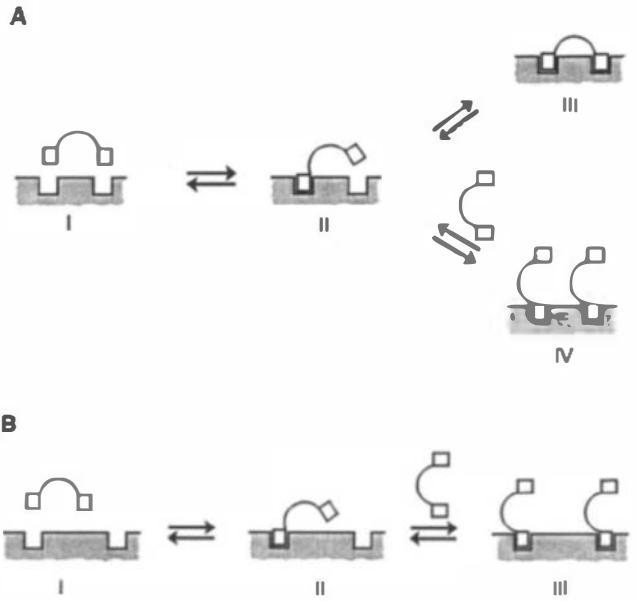
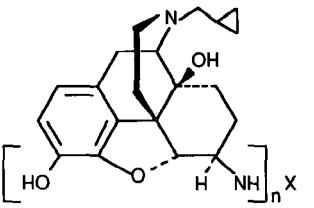


Figure 1 A diagram of the steps involved in the interaction of a bivalent ligand with neighboring recognition sites on two subpopulations (A and B) of receptors. In receptor type A the binding of bivalent ligand proceeds via the univalently bound state (II) to the totally bound state III, which is favored over occupation by two ligands (state IV). In receptor type B only univalently bound ligand (states II and III) occurs because the spacer does not permit bridging of neighboring sites.

opioid recognition sites. These neighboring sites might be located on a single opioid receptor system or on two associated receptors. The potency increase of a double pharmacophore bivalent ligand over a monovalent ligand should be substantially greater than a factor of two if the confinement of the free pharmacophore of a univalently bound ligand is held within the locus of the neighboring vacant recognition site, as this would be equivalent to a very high concentration of pharmacophore (Figure 1A, state II). Consequently, bivalent binding (state III) should be favored over univalent binding (state IV) if the spacer length permits bridging of neighboring sites. The simultaneous occupation of two recognition sites (state III) should therefore lead to selectivity if such binding is favored by a single subpopulation of receptors. The dimensions of the spacer would be expected to play an important role in modulating selectivity, as factors such as the length, geometry, and conformational mobility of the spacer should influence the orientation of the unbound pharmacophore in the univalently bound state (Figure 1, II). A

hypothetical case depicting how spacer length may modulate selectivity via the bridging principle is illustrated by comparing Figure 1A with 1B. Receptor type A is bridged by the bivalent ligand more readily than receptor type B because the spacer does not permit both pharmacophores to bind neighboring sites simultaneously in receptor type B.

The first selective κ opioid receptor antagonist, TENA, **3**, was developed out of the double pharmacophore bivalent ligand approach (9, 10). TENA consists of two naltrexone-derived pharmacophores connected to a spacer obtained from triethylene glycol. In smooth muscle preparations TENA is κ-selective, whereas its homologue **4** with a longer spacer is nonselective. The monovalent analogue **5** also is nonselective, suggesting that two recognition elements and a proper length spacer are required for κ selectivity.



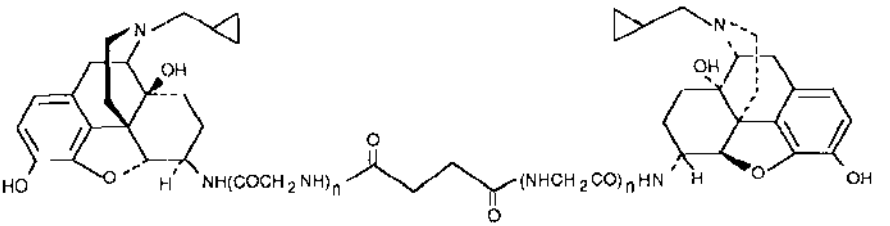
	n	X
3 (TENA)	2	$CH_2CH_2OCH_2CH_2OCH_2CH_2$
4	2	$(CH_2CH_2OCH_2CH_2OCH_2CH_2)_2O$
5	1	$CH_2CH_2OCH_2CH_2OCH_2CH_2OCH_3$

The spacers employed in subsequent studies (11, 12) are composed of glycyl units. This property permitted varying of the spacer length by the number of glycyl units and it provided facile elaboration of such spacers through standard peptide chemistry. Also, glycyl units were preferred to avoid incremental increases in the hydrophobic properties of the bivalent ligand upon lengthening the spacer. Symmetry was introduced into the spacers by a central succinyl or fumaryl group (series **6** and **7**, respectively). Both groups were employed to compare the relationship between the conformational flexibility of the spacer and antagonist potency. Monovalent ligands containing an appropriate spacer were employed to factor out possible contributions of the spacer to activity.

The structure-activity profiles **6** and **7** are presented in Figure 2. These studies were carried out on the guinea pig ileum preparation (GPI), which contains μ and κ receptors. The graphs illustrate the relative effectiveness of

members of the series to antagonize either morphine (μ-selective agonist) or ethylketazocine (κ-selective agonist) as a function of the number of glycyl units (n) in the spacer. Significantly, the structure-activity relationship profile of the succinyl series **6** for antagonism of morphine is substantially different from that of ethylketazocine (Figure 2, Panel A); peak antagonism of morphine is observed at n=2, whereas maximum antagonism of ethylketazocine is seen in the bivalent ligand having the shortest spacer length (n=0). The structure-activity results for series **6** are qualitatively consistent with the data obtained with TENA **3** and its higher homologue, in that the bivalent ligands with the shortest spacers are the more potent κ antagonists. The large increase of antagonist potency at μ receptors is consistent either with the bridging of two neighboring receptor sites or two neighboring subsites on a single μ opioid receptor.

In an effort to distinguish between these two possibilities, the bivalent ligand containing a combination of (−) and (+) enantiomeric elements (Figure 3, meso isomer) was synthesized (13). This ligand possesses the same spacer (n=2) that afforded peak antagonism at μ receptors. The (+)-enantiomer was incorporated into this molecule because it has been established that (+)-naltrexone (14) is inactive as an opioid antagonist. It was found that the meso isomer and the monovalent ligand possess nearly equal



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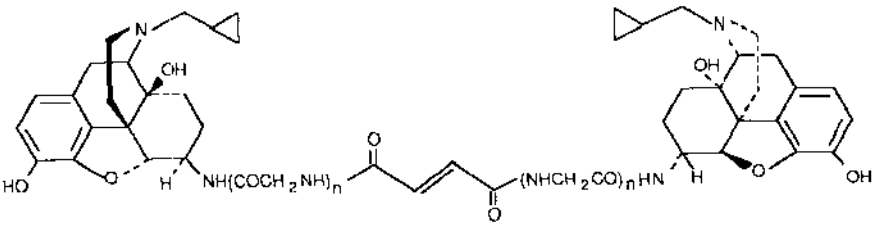

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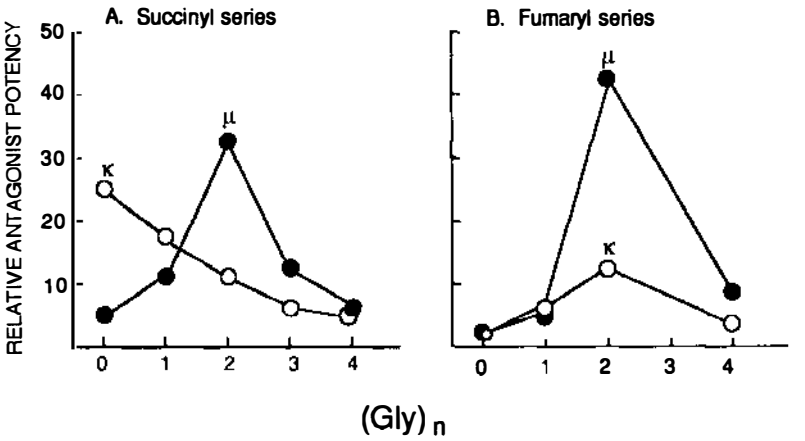


Figure 2 Relationship between relative opioid antagonist potency and the number of glycyl units, $(Gly)_n$, in each half of the spacer of bivalent ligands in succinyl series **6** (panel A) and fumaryl series **7** (panel B).

antagonist potencies, but ~1/30 less than the (−)-(−) isomer, thereby confirming that the neighboring site has an enantio-preference characteristic of an opioid receptor site.

The closely related fumaryl series **7** possesses a structure-activity-relationship profile at μ receptors similar to that of the succinyl series **6** (12). However, the interaction of series **7** at κ receptors differs substantially from that of **6** (Figure 2, Panel B). This difference is characterized by the significantly longer spacer requirement for peak κ antagonist potency in the fumaryl series relative to the succinyl series. It has been proposed that conformational restriction imposed by the fumaryl group in a short spacer (n = 0) prevents effective interaction of both pharmacophores with neighboring recognition sites of the κ receptor system. As the spacer is lengthened (n = 2) and becomes more flexible, the simultaneous occupation of neighboring sites is facilitated.

These results led to investigation of bivalent ligands with a very short, rigid spacer. This approach was based on the idea that immobilization of the antagonist pharmacophores in the proper orientation might facilitate simultaneous occupation of neighboring recognition sites. Pyrrole was used as a spacer based upon its synthetic accessibility (15) and because it should restrict conformational mobility of both pharmacophores by virtue of fusion at positions C-6 and C-7 of the morphinan structure. Two members of the series, norbinaltorphimine **8** (norBNI) and binaltorphimine **9** (BNI), possess ex-

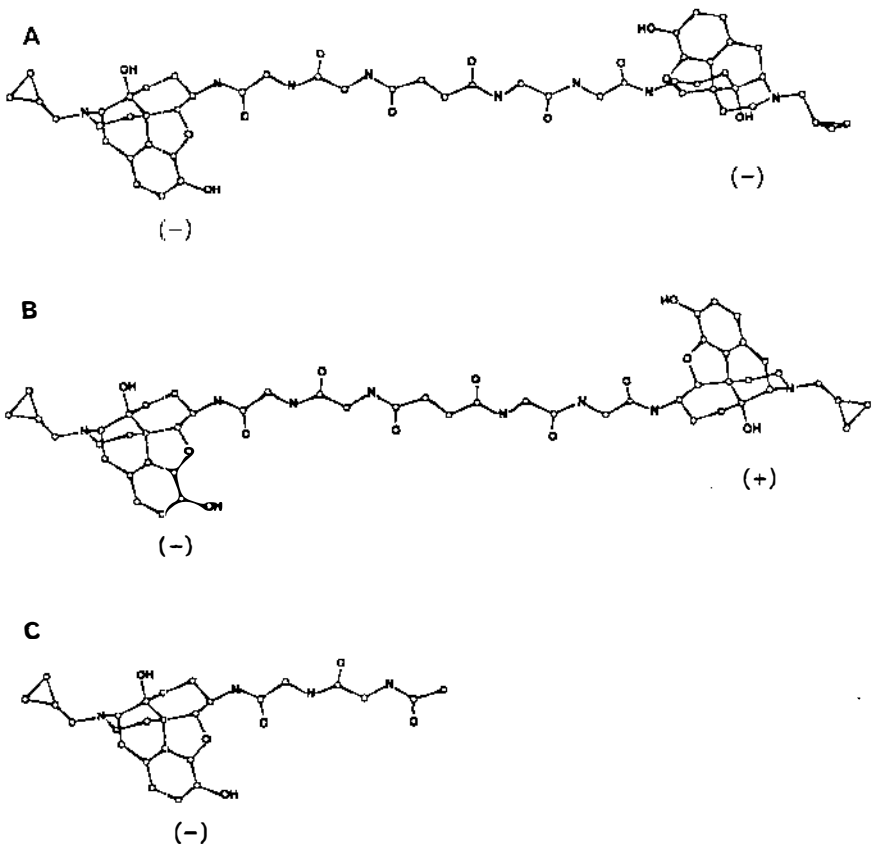
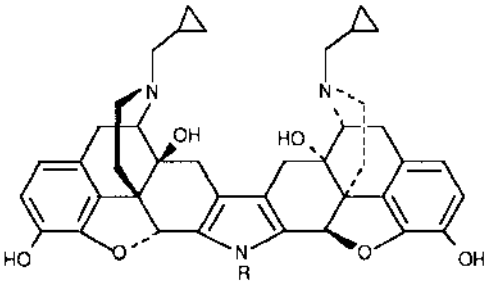


Figure 3 Comparison of a bivalent ligand containing two (−)-naltrexone-derived pharmacophores (A) with its meso isomer (B) and monovalent ligand (C).

ceptionally high κ opioid receptor antagonist potency and unprecedented κ antagonist selectivity (16). This high in vitro antagonist selectivity of norBNI is paralleled by its high binding selectivity for κ opioid sites and its κ selectivity in mice (16–18). It is noteworthy that this selectivity arises from an increased affinity at κ sites and decreased affinity at μ and δ sites.

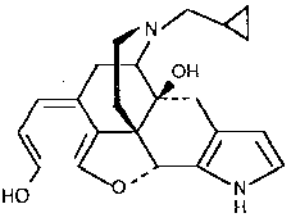
Structure-activity studies (19) suggest that the minimum requirements for κ selectivity are (*a*) at least one free phenolic OH group, and (*b*) at least one N-cyclopropylmethyl or N-allyl substituent. The fact that the 17, 17'-diacetate ester of norBNI is as potent and selective as norBNI indicated that the 14-hydroxy function is not essential for activity. The finding that the monovalent ligand **10** that contains only a fused pyrrole is not κ-selective, suggests that the pyrrole moiety functions primarily as a spacer.



8 norBNI, R = H

9 BNI, R = CH_3

As it was uncertain whether norBNI **8** derives its κ selectivity by interacting with two neighboring κ opioid receptor sites or with two subsites on a single κ receptor, the meso isomer **11** was synthesized (20). The logic for the synthesis of **11** was based on the fact that it contains a combination of the antagonist pharmacophore derived from (−)-naltrexone and its inactive (+)-enantiomer. The perspective formulas (Figure 4) corresponding to **8** and **11** illustrate the



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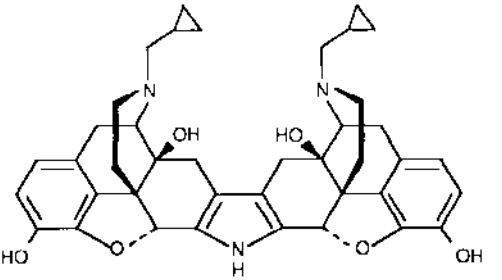

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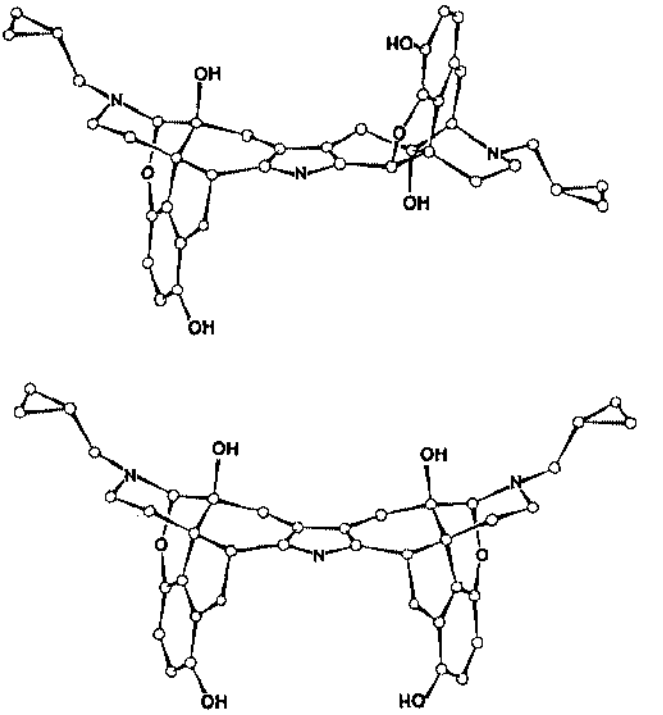
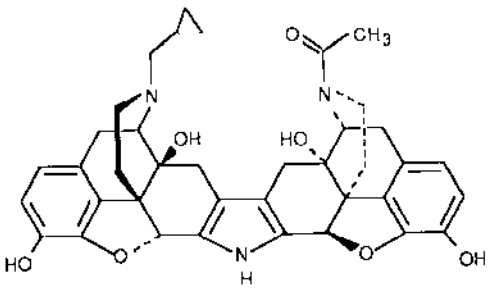


Figure 4 The stereostructures of norBNI **8** (upper) and its meso isomer **11** (lower).

different geometry of these molecules. Thus if κ opioid antagonist selectivity is retained, it could not be ascribed to bridging two neighboring opioid receptors. In smooth muscle preparations **11** is ~5 times more potent than norBNI **8** and it is κ-selective (20). This is consistent with the idea that only one of the two antagonist pharmacophores of norBNI is required for κ opioid antagonist activity and selectivity.

This study suggests that the κ opioid receptor site is comprised of two key subsites. One subsite recognizes the tyramine moiety of a single antagonist pharmacophore in norBNI, while the second subsite interacts with an element of the second pharmacophore. This second subsite was considered to be unique for κ receptors. Since the basic nitrogens of norBNI and meso-norBNI are in similar positions relative to one another (see Figure 4), it was proposed that one of the basic groups in each of these ligands mimics the Arg^7 residue of dynorphin at the κ receptor recognition site (5). This conclusion was based on studies showing Arg^7 to be essential for κ opioid activity (21).

To test this model, norBNI analogue **12** was synthesized. As **12** contains only one basic group, its κ antagonist activity should be reduced substantially.



12

Accordingly, in smooth muscle preparations, **12** possesses only 1/75th the κ antagonist potency of norBNI and is no longer κ-selective (22). Thus, the data are consistent with a model in which one of the basic groups of norBNI mimics the basic Arg^7 residue of dynorphin.

Since norBNI contains recognition units that function as an opioid antagonist pharmacophore and as a κ receptor "discriminator" (second basic nitrogen) that enhances the affinity for κ recognition sites, it is of interest to divide norBNI into functional elements. From this perspective, the rigid spacer that connects the tyramine moiety in the first half of norBNI with the basic nitrogen (κ receptor discriminator) in the second half, consists of both C rings of morphinan and one piperidine carbon (Figure 5). The most relevant segment of dynorphin is depicted below norBNI in Figure 5 to illustrate the possible correspondence of the key basic moieties in each of these molecules. From the standpoint of recognition, the κ receptor discriminator of norBNI possibly mimics Arg^7 of dynorphin, perhaps through ion pairing with an anionic group at a unique κ receptor subsite.

In an effort to explore the orientation of the discriminator subsite relative to the pharmacophore recognition site, the pyrrole moiety of norBNI was replaced with either thiophene **13** or pyran **14** (18). Both of these bivalent ligands are κ-selective; the thiophene analogue, **13,** exhibits a binding selectivity profile that resembles norBNI, whereas the pyran analogue shows considerably lower κ selectivity. The relative affinities for κ sites are norBNI **> 13 > 14.**

The differences in binding selectivities and in affinities may reflect the different orientations of the subsite discriminator due to the geometric constraint of the discriminator imposed by the spacers (Figure 6). Since the geometries of norBNI and the thiophene analogue, **13**, bear the closest resemblance, it is reasonable to conclude that they possess similar selectivity ratios. One interpretation of the data is that a hypothetical anionic group on a

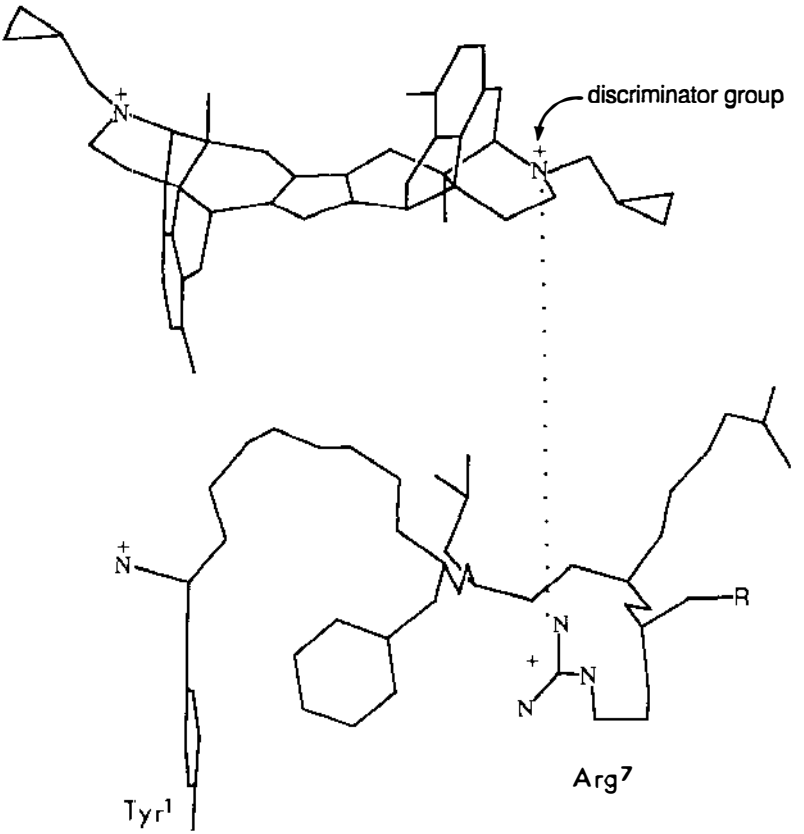
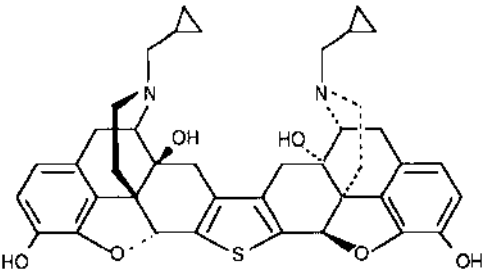


Figure 5 The relationship between key recognition elements in norBNI (upper) and those in dynorphin (lower). The second basic nitrogen (discriminator) of norBNI is aligned with that of the Arg^7 guanidinium group in dynorphin (in an arbitrary conformation) to illustrate its possible role as a key recognition element in conferring κ selectivity.

putative subsite of the κ recognition site may more easily be bridged when the heterocyclic portion of the spacer is pyrrole or an isosteric moiety.

Message-Address Approach in the Design of Selective Delta Opioid Receptor Antagonists

The model discussed in connection with norBNI **8** and related κ-selective opioid antagonists bears a formal resemblance to the "message-address" concept proposed by Schwyzer (23), who developed it to analyze the structure-activity relationship of ACTH and related peptide hormones. Accordingly, peptide hormones contain a "message" sequence and an "address" sequence of amino acid residues, each being sequential and close to one



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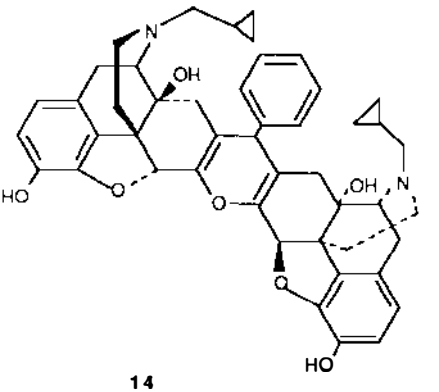
another in the peptide chain. The message component is responsible for signal transduction, while the address provides additional binding affinity and is not essential for the transduction process.

The endogenous opioid peptides appear to conform to this model in that they contain an N-terminal tetrapeptide sequence, Tyr-Gly-Gly-Phe, that is an important requirement for opioid activity (24). It has been proposed that this N-terminal tetrapeptide sequence carries the "message" responsible for mediating the opioid effect and that segments of these peptides that differ in amino acid sequence play an "address" role in conferring selectivity (21). That is, its function is to bind to a unique subsite that is complementary to each opioid receptor type.

One implication of the application of the message-address concept to opioid receptors is that the message subsite is invariant or very similar for all receptor types in a class and that the address subsite is the primary determinant of selectivity among receptor types and subtypes. It is important to point out that this model should not be viewed too literally, as a small portion of the message and address elements may possibly be confluent with respect to



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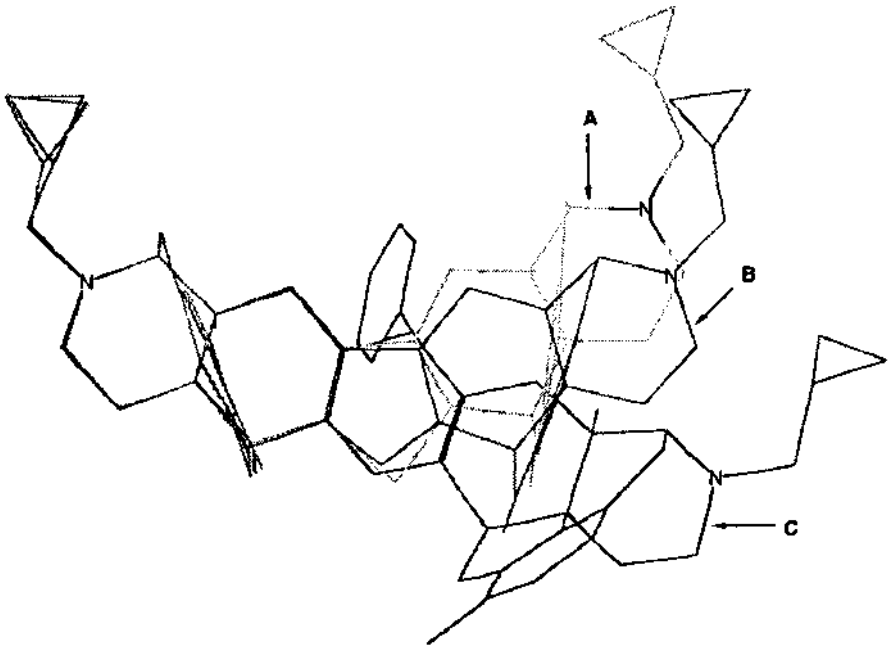


Figure 6 Superposition (top view) of norBNI **8** (B) with its thiophene **13** (A) and pyran **14** (C) analogues.

function. Diagrams for selection among different receptor types are presented to illustrate this concept (Figure 7).

Studies directed toward testing the message-address concept with respect to opioid receptor selectivity reveal that a typically μ-selective ligand, oxymorphone, can be transformed to a δ-selective ligand simply by attachment of a "δ address" (Phe-Leu) through a spacer to the C-6 position of the opiate (Figure 8; 25). Similarly, a κ-selective ligand was synthesized by attachment of a "κ address" (Phe-Leu-Arg-Arg-Ile-OMe). Although the δ and κ binding are relatively low (selectivity ratios fo 2–15), they represented a dramatic change from that of the unsubstituted semicarbazone (A=NH_2; Figure 8), which is μ-selective (selectivity ratios of 5–12). These results suggest that the Tyr^1 residue of the opioid peptides comprises the message component and the sequence starting with Phe^4 constitutes the address; in this context, Gly^2-Gly^3 serves as a spacer. This is consistent with the well-known structure-activity relationships of nonpeptide opioid ligands (e.g. morphine or oxymorphone) that contain only one aromatic ring that presumably mimics the Tyr^1 residue. The C ring of the morphinan structure and semicarbazone moiety in these hybrid molecules may serve as a mimic for the Gly^2-Gly^3 spacer. Alternately,

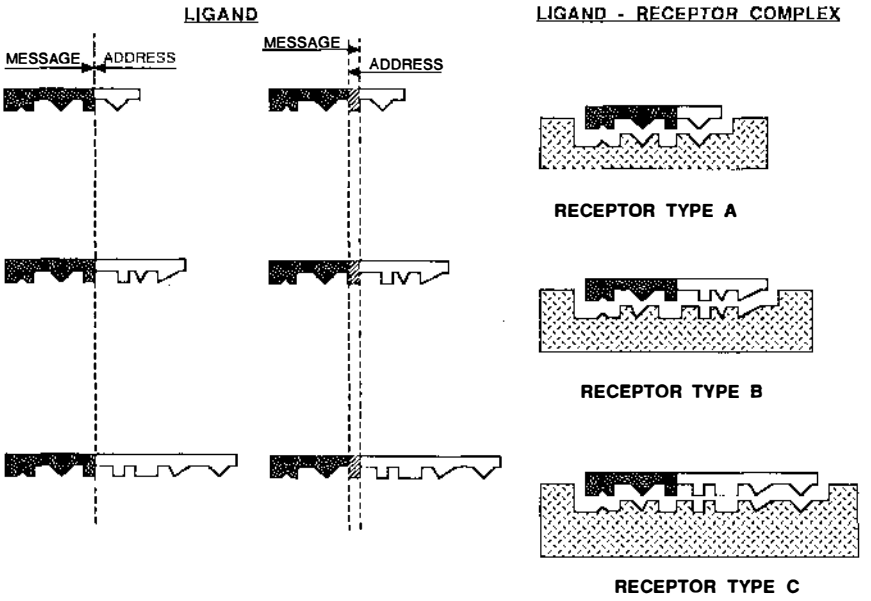
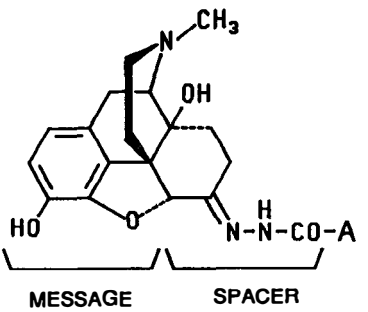


Figure 7 A cartoon of the message-address concept. The ligands contain a message and an address sequence of amino acid residues in the peptide chain. The second column represents ligands whose message and address sequences overlap (hatched area) to a small extent.



A (ADDRESS)	SELECTIVITY
Phe-Leu-OH	delta
Phe-Leu-Arg-Arg-Ile-OMe	kappa

Figure 8 Hybrid structures derived from oxymorphone and the key "address" segments of enkephalin (δ) or dynorphin (κ).

it is conceivable that the Phe residue in both of the "address" components may serve both as a functional but nonessential part of the message and as an essential part of the address. That is, there is overlap in the functional recognition elements of the hybrid ligands (see Figure 7).

Since the structure-activity relationship studies of norBNI-related structures and the opiate-peptide hybrids suggest that their selectivities are consistent with a message-address model, the design of nonpeptide δ opioid receptor antagonists was undertaken. Although the concept of a message and an address was proposed for peptide agonists, it has served as a useful model for the design of antagonists based on the premise that such ligands also interact with the same message and address subsites. An important consideration in this design was the conformational restriction of the nonpeptide address moiety, as this would preclude possible conformational adaption in the binding to other opioid receptor types. In fact, the relatively low binding selectivity of endogenous opioid peptides (24) may be a consequence of such conformational adaption due to their flexible nature.

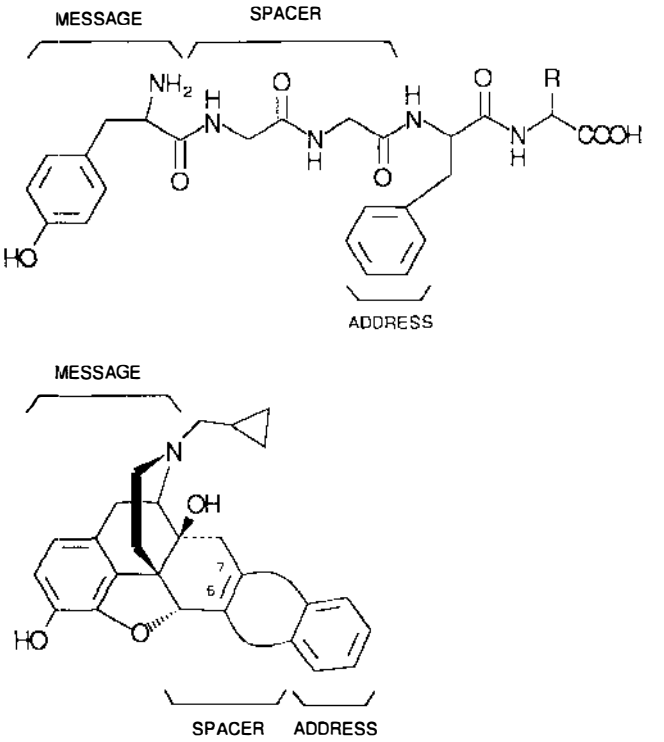
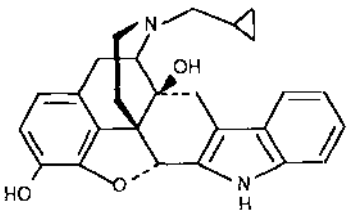
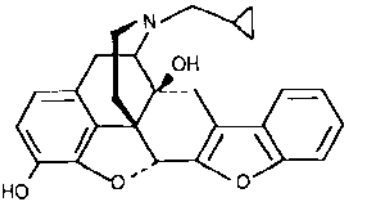


Figure 9 The relationship between key recognition elements of leucine enkephalin and those of a non-peptide δ opioid antagonist that contains a δ address mimic.



15 (NTI)

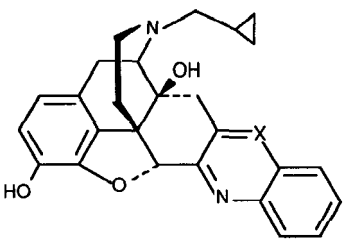


16 (NTB)

The design strategy for nonpeptide, δ-selective antagonists employed a naltrexone-derived structure for the message moiety and a key element of the leucine enkephalin δ address component (8, 26). This element, which was hypothesized to be the benzene moiety of Phe^4, was fused to the morphinan structure of naltrexone through a rigid spacer. The relationship of the functional components of the nonpeptide to leucine enkephalin is illustrated in Figure 9.

The first target compound contains a pyrrole spacer because it was easily accessible from naltrexone in a single synthetic step that permitted quick access to the target compound in order to test the model. This compound, naltrindole **15** (NTI), is the first reported (26, 27) nonpeptide δ-selective opioid receptor antagonist. The δ antagonist potency in vitro was about 500 times greater than the δ-selective enkephalin analogue, $(allyl)_2$Tyr-Abi-Abi-Phe-Leu-OH (ICI174864). In terms of binding, NTI possesses over a 1000-fold greater affinity for δ opioid receptors than ICI174864 (8). The profound effect of the address moiety in NTI is also demonstrated by its 240-fold greater δ antagonist potency over its precursor, naltrexone (8).

Since the pyrrole moiety functions as a spacer, other heterocycles can play a similar role. Thus, replacement of pyrrole with furan affords the benzofuran analogue **16** (NTB), which possesses half the δ antagonist potency and tenfold greater affinity than NTI (28). Analogues with 6-membered heterocyclic spacers **17** are δ-selective, but substantially less potent and selective than NTI



17 X = CH or X = N

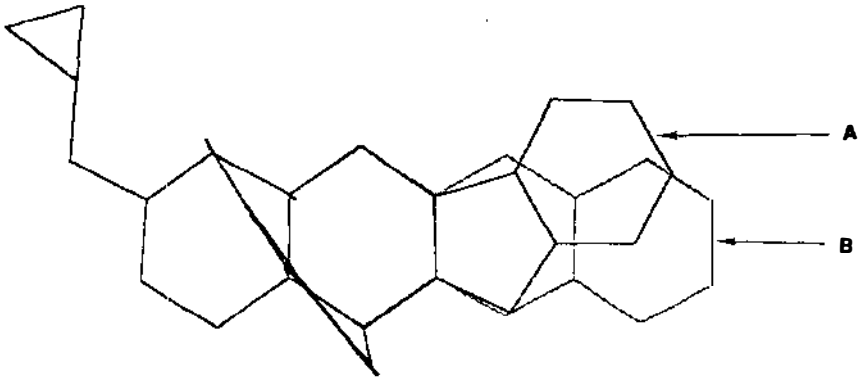
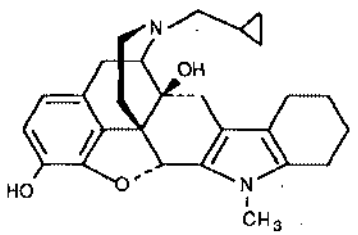


Figure 10 Superposition (top view) of NTI **15** (A) upon its analogue **17** (B).

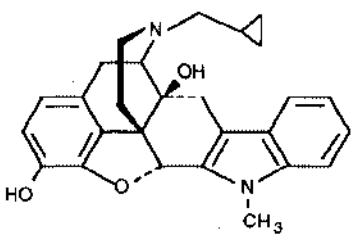
(28). This may be due to the different geometry of the spacer, as a 6-membered ring orients the address mimic (benzene moiety) differently from a 5-membered ring, as illustrated by the superposed structures (Figure 10). Consequently, it may not interact as well with the address subsite.

Significantly, the finding that N-methyl-NTI **19** is ninefold more potent than its saturated derivative **18** (28) is consistent with the decreased potency of enkephalin analogues that contain a hexahydro-Phe[4] residue (29). This finding lends additional support to the similar roles of Phe[4] the indolic benzene moiety.

It is noteworthy that compound **18** is apparently as δ-selective as its indole counterpart despite its lower antagonist potency. This illustrates an important point concerning the design of selective ligands; namely, that molecular modification of a highly selective ligand that leads to a concomitant proportional decline in the potency at all three receptor types can afford a congener with its selectivity retained.



18



19

SELECTIVE ANTAGONISTS AS PHARMACOLOGICAL PROBES

Kappa Opioid Receptor Antagonists

The first selective κ opioid receptor antagonist, TENA (**3**), has been shown to be about 5 and 27 times more potent in antagonizing the effects of ethylketazocine (EK) and trans-(±)-3,4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl)-cyclohexyl]benzeneacetamide (U-50,488H), respectively, than that of morphine in the guinea pig ileal longitudinal muscle preparation (GPI)(10). TENA possesses much higher selectivity for κ opioid receptors than the putative κ receptor antagonists, MR 2266 or WIN 44,441, which possess κ/μ selectivity ratios of 1.2 and 0.3, respectively. By comparison, naloxone in the GPI displays a κ/μ selectivity ratio of only 1.2. In addition, when the abilities of ligands to protect κ receptors from alkylation by β-chlornaltrexamine (a universal, nonequilibrium opioid antagonist) (30) in the κ-enriched GPI (GPI depleted of functional μ receptors with β-funaltrexamine) were examined (31), the results revealed that TENA is considerably more effective than naloxone or κ receptor agonists such as EK and U-50,448H. TENA has not been employed as a pharmacologic tool to study κ opioid receptor-mediated effects because it was soon superseded by the more selective and potent κ opioid receptor antagonists, binaltorphimine (BNI) and norbinaltorphimine (norBNI) (16, 32).

The highly potent and selective κ opioid receptor antagonists, norBNI **8** and BNI **9,** (16) possess Ke values in the sub-nM range when tested against EK and U-50,488H in the GPI and against EK in the rabbit vas deferens, a preparation that contains only κ opioid receptors (33). The pA_2 values for the antagonism of U-50,488H by norBNI in the GPI and mouse vas deferens (MVD) preparations have been reported to be over 10 (34). Similar pA_2 values have been reported for the antagonism by norBNI of the effects of EK, dynorphin (1–17), tifluadom, and bremazocine in the GPI (35). In contrast, the pA_2 values for the antagonism of [D-Ala^2,MePhe^4,Gly-ol^5]enkephalin (DAMGO) and [D-Pen^2, D-Pen^5]enkephalin (DPDPE) by norBNI in the MVD are 7.6 and 7.8, respectively (34), which represent several hundredfold less affinity of norBNI for μ and δ receptors.

When BNI is administered either i.c.v. or parenterally to mice, it inhibits substantially the antinociceptive activity of κ receptor agonists, EK and U-50,488H, at doses that have no influence on the antinociceptive effect of the μ receptor agonist, morphine, or that of the δ receptor agonist, [D-Ser^2, Leu^5]enkephalin-Thr (DSLET) (16). NorBNI has a similar κ-selective profile in vivo wherein the antinociceptive effects of EK and U-50,488H are prominently antagonized while those of morphine, DAMGO and DPDPE are unaffected (17). Recently norBNI has been shown to possess unusually long

antagonistic effects lasting several days (36) to weeks (F. Porreca, personal communication). In opioid receptor binding assays, norBNI possesses over a 150-fold selectivity for κ over μ and δ receptor sites (17). The latter report indicated that BNI does not have very good κ selectivity but it was subsequently found to display κ selectivity comparable to that of nor-BNI (18).

With the availability of norBNI, it was now possible to examine directly the relative involvement of κ opioid receptors in many pharmacologic and physiologic effects. The first pharmacologic effect that was investigated was the antinociception induced by κ opioid receptor agonists. NorBNI was used in mice to show that the most important site for the involvement of κ opioid receptors in antinociception is the spinal cord (17, 37), although antinociception could occur solely by interaction at κ opioid receptors in the brain both in mice (17) and rats (38). The best evidence for the importance of spinal κ receptors in mediating antinociception is that i.t. administered norBNI is many times more sensitive than i.c.v. administered norBNI in antagonizing the effects of parenterally administered U-50,448H (17). NorBNI also has been employed to demonstrate that κ opioid receptors are involved in the mediation of antinociception induced by nitrous oxide (39), corticotropin-releasing factor (CRF) (40), psychological stress (41), and pregnancy (42). Presumably, release of endogenous opioid peptides, possibly dynorphins, are involved in the mediation of these antinociceptive effects.

When administered i.t. in mice, CRF has been shown to antagonize the antinociceptive activity of morphine and it has been postulated that this antagonistic effect is mediated through the release of dynorphins (43). In this case, the authors have demonstrated that CRF does release immunoreactive dynorphin A from isolated spinal cords in a superfusion system (44) and both dynorphin (1-17) and dynorphin (1-8) have been shown to antagonize the antinociceptive effect of morphine when administered i.t. (45). The antagonistic effect of the dynorphins could be blocked completely by norBNI or α-helical CRF (9-41), a CRF receptor antagonist, which suggests that the antagonistic effect of CRF involves both CRF and κ opioid receptors.

Recently, it was found that naloxone produces protracted tolerance to morphine in mice, i.e. if naloxone is administered several hr or even up to 3 days after an acute tolerance-inducing dose of morphine (which produces acute tolerance lasting <24 hr), the tolerant state can be maintained for over two weeks (46). Because large doses of naloxone were required to produce this effect, the involvement of μ opioid receptors was considered unlikely. Thus, norBNI was used to investigate the possible involvement of κ opioid receptors (47). NorBNI was found to mimic the effects of naloxone, i.e. the selective blockage of κ opioid receptors leads to the protractive maintenance of acute morphine tolerance. In addition, norBNI has been shown to greatly

exacerbate the expression of acute tolerance when it is administered just before antinociceptive testing (48).

The release of several neurotransmitters and hormones appear to be regulated through κ opioid receptors. [^{3}H]Dopamine release that has been stimulated either by K^+ in striatal and cortical slices of rats and guinea pigs (49) or electrically in slices of nucleus accumbens, olfactory tubercle, and frontal cortex of rats (50), is inhibited by U-50,488H and this inhibition is completely antagonized by norBNI. Dopamine release does not appear to be influenced by interactions at either μ or δ opioid receptors. NorBNI and U-50,488H also have been used to demonstrate that activation of κ opioid receptors inhibits all dopaminergic neuronal systems in the brain (51). U-50,488H and norBNI have been shown to inhibit and activate, respectively, the tuberohypophysial dopaminergic neurons, which project to the neural and intermediate lobes of the pituitary gland (51, 52). The regulation of the secretion of α-melanocyte-stimulating hormone is thought to act through this mechanism, i.e. activation of κ opioid receptors inhibits the activity of tuberohypophysial dopaminergic neurons that, in turn, increases the secretion of α-melanocyte-stimulating hormone from the intermediate lobe of the pituitary gland (53).

Histamine release from brain slices of rats also appears to be regulated specifically through κ opioid receptors (54). The K^+-stimulated release of [^{3}H]histamine from brain slices labeled with [^{3}H]histidine is inhibited by a number of κ opioid receptor agonists such as ketazocine, dynorphin A(1-13), spiradoline, U-50,488H, and U-69,593, but not by several selective μ or δ opioid receptor agonists. The inhibitory effects on histamine release by the various κ receptor agonists have been shown to be reversed by norBNI much more potently than by naloxone. In contrast to dopamine and histamine release, regulation of norepinephrine release appears to involve not only κ but μ and δ opioid receptors as well (55). The K^+-stimulated release of [^{3}H]norepinephrine from cortical slices of guinea pigs is inhibited by the μ-, κ-, and δ-receptor selective agonists, DAMGO, U-50,488H, and DPDPE, respectively. NorBNI suppresses the inhibitory effect of U-50,488H but has little effect on those of DAMGO or DPDPE.

The release of opioid peptides from the hypothalamus of rats has been postulated to regulate their own release through interaction at presynaptic auto-receptor sites by inhibitory negative feedback mechanisms (56). Although norBNI increases the release of only dynorphin, the δ receptor antagonist, ICI 174864, enhances the release of all three types of opioid peptides, namely β-endorphin (predominantly μ), dynorphin (κ), and met-enkephalin (δ). The μ receptor antagonist, D-tetrahydroisoquinoline-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH_2 increases the release of β-endorphin and dynorphin. Thus, there appears to be a cross-regulation of release of the three opiopeptins. The authors suggested the term, "allelo-receptors" as opposed to

auto-receptors in cases where an endogenous peptide interacts not only with its own specific receptor to regulate its release but also interacts with other receptors of related peptides to regulate their release.

Endogenously released opioid peptides suppress the pulsatile release of luteinizing hormone (LH) in early gestation in the rat, and this inhibition can be blocked by naloxone. To determine the opioid receptor type involved in this effect, selective antagonists, norBNI (κ), β-FNA (μ), and ICI 174864 (δ) have been utilized (57). Only norBNI exerts a stimulatory action on the release of LH. Thus, the regulation of the release of LH in early pregnancy in rats appears to involve κ opioid receptors.

Reports of the beneficial effects of opioid antagonists in spinal cord injury and the resulting ischemia and paralysis have been controversial. Because i.t. administered dynorphin (1-13) mimics the paralysis due to spinal injury, κ opioid receptors were implicated in this condition (59). An early report demonstrated the efficacy of norBNI in significantly improving neurological recovery after spinal trauma in rats (60). However, this finding was not corroborated by others (61). Later, it was found that dynorphin fragments with no opioid activity such as dynorphin (3-13) and dynorphin (2-17) (59, 62) and other neuropeptides such as somatostatin and its analogues and a substance P analogue (63) also produce paralysis in rats. Thus, there may be a non-opioid component as well as an opioid component that contributes to the neurodeficit in spinal cord injury (59). Whether or not opioid receptor antagonists and particularly κ opioid receptor antagonists would be useful in treating spinal cord injury becomes tenuous, especially in light of mounting evidence that κ opioid agonists rather than antagonists are highly beneficial as neuroprotectants in ischemic conditions (64-66).

Another intriguing finding that may involve κ opioid receptors and may have clinical significance in the control of obesity is the report that naloxone inhibits food and fluid intake in rats (67, 68) whereas dynorphin (1-13), dynorphin (1-17) and U-50,488H increase food and water intake (69, 70). To study the involvement of κ opioid receptors in feeding behavior, the effect of norBNI on electrically induced feeding in rats has been examined (71). Lateral ventricular injections of norBNI enhance the stimulation threshold for inducing feeding behavior whereas mesopontine aqueductal injections of norBNI decrease the feeding threshold. Thus it appears that endogenously released opiopeptins that interact with κ receptors in the forebrain facilitate feeding behavior while their interaction at κ receptors in the brain stem results in inhibition of feeding behavior. Consequently, it is likely that oral or parental administration of κ opioid receptor antagonists will not give unequivocal results with respect to the overall feeding behavior. More recently, i.c.v. administered norBNI has been shown to inhibit nocturnal food intake, short-term intake of high-fat diet, and 2-deoxy-D-glucose-induced hyperpha-

sia in rats (72). In another recent study, feeding induced by U-50,488H or deprivation in rats has been shown to be inhibited by i.c.v. administered norBNI (73). However, feeding induced by the μ- and δ-selective agonists, DAMGO and DSLET, respectively, are also inhibited by norBNI. The feeble effect of norBNI on deprivation-induced feeding may mean that κ opioid receptors are not greatly involved in natural feeding. In this regard, efforts to decrease body weight in humans as well as in laboratory animals by chronic administration of opioid receptor antagonists have been disappointing (74).

Other pharmacologic effects that have been investigated with the use of norBNI include the anticonvulsant effect of κ opioid receptor agonists, U-50,488H and PD117302 (75, 76), the antitussive effects of U-50,488H and U-62,066E on capsaicin-induced cough reflex in rats (77), diuresis produced by κ opioid receptor agonists, ethylketazocine, tifluadom, bremazocine and U-50,488H in rats (78), and the generation of antibodies by lymphoid cells of mice (79). Biochemical studies that have used norBNI to determine the involvement of κ opioid receptors include inhibition of adenylyl cyclase activity in guinea pig cerebellar membranes (80), phosphoinositide turnover in rat brain (81), and lowering of free calcium concentrations in guinea pig cerebellar synaptosomes (82). A number of papers in which norBNI was used to show the noninvolvement of κ opioid receptors have been published.

An affinity chromatography column has been prepared by coupling an analogue of norBNI, aminoethyl-norBNI (AE-norBNI), to activated agarose gel (83). When P_2 fractions of guinea pig brain are solubilized and processed through the affinity column, a receptor protein is obtained that binds with κ opioid receptor agonists, ethylketazocine, U-50,488H and dynorphin A and with the κ receptor antagonist, norBNI. The isolated receptors do not appear to bind with the μ receptor agonist, DAMGO, the δ receptor agonist, DPDPE, or the potent δ receptor antagonist, naltrindole. This column should be much more stable than those utilizing peptide analogues and should find utility in purification of κ opioid receptors.

Delta Opioid Receptor Antagonists

Peptide antagonists with high selectivity for δ opioid receptors, such as ICI 154129 and ICI 174864, have been available for several years (7). However, the utility of these antagonists was limited because they could not be administered systemically to animals due to their limited accessibility to the central nervous system. Also, ICI 174864 has been reported to display neurotoxic effects (84). The nonpeptide, δ opioid receptor antagonist, naltrindole **15** (NTI) (27), antagonizes potently the activity of the δ opioid receptor agonists, DADLE and DPDPE, with K_e values in the range of 0.1–0.3 nM when tested in smooth muscle preparations (MVD and GPI). In contrast, the K_e values for the antagonism of the activities of μ, κ, and ϵ opioid receptor agonists are at

least 100-fold greater. By comparison, the selective, peptide antagonist, ICI 174864, antagonizes the effect of DADLE with a K_e of 68 nM. In receptor binding assays, NTI is again highly selective and potent against δ opioid receptor binding with a K_i of 31 pM, which is more than three orders of magnitude lower than those of ICI 174864 and naltrexone (8, 85). The selectivity and potency of NTI in the MVD and receptor binding assays have been corroborated by other investigators (86). In vivo, NTI, administered s.c., antagonizes the antinociceptive action of DSLET in mice at doses that do not alter the effects of morphine or U-50,488H (85). The δ-selective profile of NTI in vivo has been confirmed in rats (87). NTI represents the first highly selective δ opioid receptor antagonist that is useful upon peripheral administration.

Soon after the report of NTI, other derivatives of NTI were synthesized including N-rnethyl NTI (N-Me-NTI) **19** and the benzofuran derivative, NTB **16** (26). Both of these derivatives have similar δ-selective profiles as that of NTI with K_e values about 1 nM for N-Me-NTI and 0.27 nM for NTB in antagonizing the activity of DADLE in smooth muscle preparations (28). In receptor binding assays, NTB competes for δ opioid binding sites with a K_i of 13 pM, which is about 1,500 and 12,000 times the affinity for δ receptors than for μ and κ receptors, respectively. N-Me-NTI also is highly potent and selective and displays a K_i of 18 pM in competing for δ sites. Both antagonists, administered i.c.v. or s.c., inhibit substantially the antinociceptive activity of DSLET at doses that have no influence on the antinociceptive activities of morphine or U-50,488H (86). Most notably, when the antagonists are administered s.c., NTB is the most potent antagonist and is about 26 and 39 times more potent than NTI and N-Me-NTI, respectively.

It must be cautioned that at doses much higher than those to demonstrate antagonism, the δ-selective antagonists display antinociceptive activity. For example, N-Me-NTI, NTI, and NTB are 1/2, 1/8, and 1/12, respectively, as potent as DSLET or DADLE when tested in the mouse-writhing assay (85). The agonism appears to be mediated by interaction at κ opioid receptors because their activities are inhibited most potently by norBNI (authors' unpublished observations). These compounds therefore can be considered new types of mixed agonist-antagonist analgesics in which they are antagonists and agonists at δ and κ opioid receptor sites, respectively.

When δ opioid receptor agonists other than DSLET were used to further characterize the selectivity of NTB, the antinociceptive effects of DPDPE and DADLE were unexpectedly shown to be unaffected at doses of NTB that antagonize substantially the antinociceptive effect of DSLET (88). This differential antagonism is more prominent at spinal than at supraspinal sites, i.e. when the antagonism by NTB, administered s.c., is examined after the agonists are administered i.t. and i.c.v. When cross-tolerance between

DSLET and DPDPE was studied by i.c.v. injection of a single large tolerance-inducing dose of either peptide, no apparent cross-tolerance develops between the two peptides. Based on these results, it was concluded that the antinociceptive action of DSLET and DPDPE may be mediated by different receptors, possibly δ opioid subtypes. In a related study, the existence of δ opioid subtypes was proposed based on the differential antagonism by the antagonists, naltrindole-5'-isothiocyanate (5'-NTII) and [D-Ala^2, Leu^5, Cys^6]enkephalin of the antinociceptive effects of DPDPE and deltorphin II (89). Further evidence for the heterogeneity of δ opioid receptors comes from opioid receptor binding studies (90, 91). DADLE is significantly more potent in competing for [^{3}H]DADLE and [^{3}H] DPDPE binding sites than for [^{3}H]DSLET binding sites. Also, DPDPE is more potent in competing for the binding of [^{3}H]DADLE and [^{3}H]DPDPE than for the binding of [^{3}H]DSLET. DSLET competes equipotently against the binding of all three peptides (90). Another study investigated the competition by DPDPE of [^{3}H]deltorphin II binding in brain membranes of rats and found that the competition curve fits best to a two-site model (91).

Although μ opioid receptors have been shown to play a major role in the development of opiate tolerance and dependence (92–94), the involvement of δ opioid receptors in the development of these adaptive phenomena is not as well documented. Because there have been reports that demonstrate the interaction of μ and δ opioid receptors in vivo (95, 96) as well as in vitro (97–100), NTI and its affinity label analogue, 5'-NTII (101), were used to study the involvement of δ opioid receptors in the development of opiate tolerance and dependence (102). Development of morphine tolerance and dependence is markedly suppressed by the administration of NTI or 5'-NTII before and during morphine treatment. These effects are produced by NTI and 5'-NTII at dosages that do not block the antinociceptive effects due to interactions at μ opioid receptors. Therefore, the selective blockage of δ opioid receptors prevents the development of morphine tolerance and dependence without compromising the antinociceptive activity of morphine.

Aside from the demonstrated antinociceptive effect produced by agonist interaction at δ opioid receptors, spinally and supraspinally, NTI has been employed to demonstrate that δ opioid receptor are involved in the antinociceptive effects of cholecystokinin octapeptide in mice (103) and in swim-stress-induced antinociception in adult rats (104, 105). Curiously, heroin, which one thinks of as morphinelike and a μ opioid receptor agonist, can act as a δ opioid agonist in some strains of mice (106). It has been demonstrated that heroin-induced antinociception in Swiss Webster mice is inhibited by NTI but not by the μ-selective opioid receptor antagonist, β-funaltrexamine (β-FNA), whereas in ICR mice, heroin-induced antinociception is inhibited by β-FNA but not by NTI. These results suggest that heroin mediates

antinociception by interacting at δ opioid receptors in Swiss Webster mice and at μ opioid receptors in ICR mice.

NTI, β-endorphin, and ACTH have been shown to significantly suppress mitogen-stimulated Peyer's patch lymphocytic immunoglobulin production of IgA, IgG, and IgM isotypes (107). Treatment of mice leukocytes with CRF and arginine vasopressin enhances natural killer cell activity and NTI as well as naloxone have been shown to block this effect (108). These findings suggest that δ opioid receptors are involved in the modulation of certain functions of the immune system.

SUMMARY AND CONCLUSIONS

Progress in opioid research relies heavily on ligands as probes to evaluate selectivity of action. The design of such ligands using naltrexone as a precursor has afforded a number of highly selective antagonists. These include the κ opioid receptor antagonist, norBNI, and δ opioid receptor antagonists, NTI and NTB. The unifying concept in the development of these antagonists was the enhancement of selectivity through simultaneous occupation of two neighboring recognition sites by a single ligand. These selective naltrexone-derived antagonists have been used widely to study the involvement of κ and δ opioid receptors in a variety of pharmacologic, physiologic, and biochemical effects.

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