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Medicinal Chemistry Award Address

An Approach to the Design of Receptor-Type-Selective Non-Peptide Antagonists of Peptidergic Receptors: δ Opioid Antagonists†

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Introduction

Rather than presenting a survey of research conducted in my laboratory over the years, I would like to discuss a subject of current interest—the design of selective peptidomimetic ligands. Interest in peptidomimetics is reflected by the increasing frequency of publications on this subject and the fact that it is one of the topics at this symposium.[1]

I am aware there are many definitions of peptidomimetics. For the purpose of this lecture, I define peptidomimetics as non-peptide ligands that are recognized by peptide recognition sites. This broad definition includes a range of structural classes of compounds—from alkaloids that bear little resemblance to peptides to non-peptides with a closer structural relationship to endogenous peptides. As this definition focuses on recognition rather than function, it includes both agonists and antagonists.

Because peptides are metabolically labile and have problems being orally absorbed, the design of peptidomimetics is being actively pursued in many laboratories. There are a number of non-peptide drugs now in clinical use that are known to interact with peptide recognition sites. I would guess that as our knowledge base of receptors and enzymes expands, the number of known drugs classified as peptidomimetics will increase.

A number of peptidomimetics that bear only a remote structural resemblance to the native peptide have been synthesized.[1] Invariably these have come from natural products or from synthetic compounds uncovered in screening programs, as it is not clear how such peptidomimetics are recognized by peptide recognition sites. The crux of the problem is that in most cases medicinal chemists are handicapped by the lack of detailed information on the molecular structure of the peptide recognition sites. What is promising, however, is the fact that relatively small non-peptide molecules can be recognized by sites that bind larger endogenous peptides.[2] This suggests that it may be possible ultimately to design small non-peptide molecules as receptor antagonists or agonists.

In this lecture I present some of our recent efforts to design δ-selective opioid receptor antagonists. Perhaps some of the principles discussed here may have sufficient generality to be useful in the design of non-peptides for peptidergic recognition sites other than those located on opioid receptors.

Multiple Opioid Receptors

The concept of multiple opioid receptors emanated from two convergent lines of research. The proposal for multiple receptors and multiple modes of interaction of ligands with opioid receptors was originally suggested on the basis of structure–activity analysis of several series of opioid agonists.[3,4] This was followed by pharmacological studies which led to a detailed framework for this concept.[5-10]

† This is in part taken from the text of the Medicinal Chemistry Award Address delivered on July 30, 1990 at the 22nd National Medicinal Chemistry Symposium, July 29–August 3, Austin, Texas.

(1) Morgan, B. A.; Gainor, J. A. Approaches to the Discovery of Non-Peptide Ligands for Peptide Receptors and Peptidases. *Annu. Rep. Med. Chem.* **1989**, *24*, 243–252.
(2) A classical example is morphine which can mimic the effect of the 31 amino acid opioid peptide β-endorphin, at a common recognition site. See: Yamashiro, D.; Li., C. H. β-Endorphin: Structure and Activity. In *The Peptides*; Udenfriend, S., Meienhofer, J., Eds.; Academic Press: New York, 1984; Vol. 6, pp 191–217.
(3) Portoghese, P. S. A New Concept on the Mode of Interaction of Narcotic Analgesics with Receptors. *J. Med. Chem.* **1965**, *8*, 609–616.
(4) Portoghese, P. S. Stereochemical Factors and Receptor Interactions Associated with Narcotic Analgesics. *J. Pharm. Sci.* **1966**, *55*, 865–887.
(5) Martin, W. R. Opioid Antagonists. *Pharmacol. Rev.* **1967**, *19*, 463–521.

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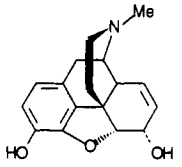
Presently, there are a minimum of three major opioid receptor types (μ, κ, δ) that are involved in the modulation of a variety of physiological effects via interaction with opioid peptides.[11] The opioid peptides enkephalin and dynorphin are believed to be the endogenous ligands for δ and κ receptors, respectively. A mammalian opioid peptide that is selective for μ receptors has not yet been identified.

A variety of non-peptide opioid agonist ligands are known to be selective for μ- or for κ-receptors. Among these, morphine (1) and ethylketazocine (2) are proto-



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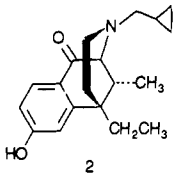

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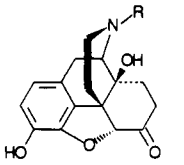
typical ligands that are selective for μ and κ sites, respectively. Various enkephalin analogues are selective for δ receptors. These include [D-Ala^2,D-Leu^5]enkephalin (3, DADLE) and later generations of more selective synthetic enkephalin-related peptides. Highly selective opioid agonists for all three sites are now known.[12]

Opioid Antagonists as Pharmacological Tools

Opioid antagonists have been indispensable as tools in opioid research.[12] In fact, the chief criterion for the classification of an agonist effect as being opioid receptor-mediated is the ability of naloxone (4) or naltrexone



4 R = $CH_2CH{=}CH_2$

5 R = $CH_2CH(CH_2)_2$

(5) to reversibly antagonize this effect in a competitive fashion. The usefulness of naloxone and naltrexone for this purpose stems from the fact that they are universal opioid antagonists; that is, they are capable of antagonizing the agonist effects mediated by multiple opioid receptor types.

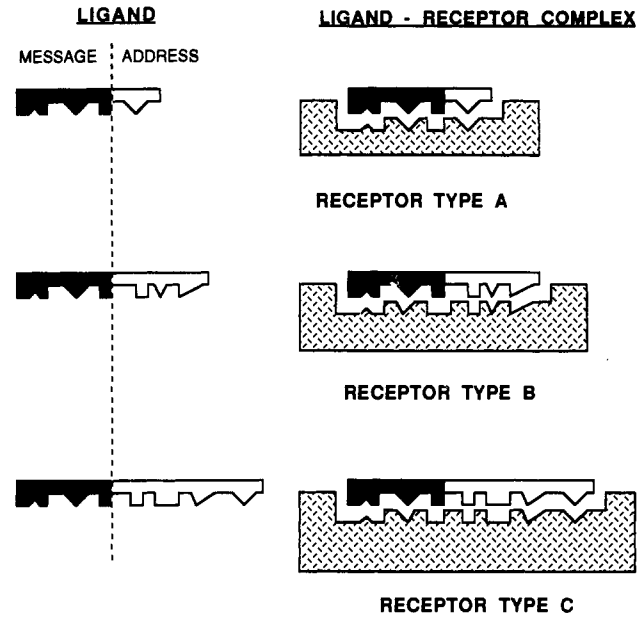


Figure 1. A cartoon of the message–address concept as a basis for the selectivity of a family of sychnologically organized peptides.

Since it is now firmly established that there are a minimum of three opiod receptor types, it has become increasingly evident that selective opioid antagonists are valuable pharmacological tools for identifying receptor types involved in the interaction with opioid agonists. One of the major advantages of selective opioid antagonists over selective agonists is their utility in probing the interaction of endogenous opioid peptides and new opioid agonists with opioid receptor types. Moreover, since it is sometimes not easy to distinguish among μ, δ, and κ opioid receptor mediated agonist effects if the pharmacological endpoints are identical (e.g., antinociception or inhibition of a smooth muscle preparation by agonists), selective antagonists clearly have wider utility as tools than selective agonists.

An aspect of selective antagonists that deserves mention is that their general utility as pharmacological tools depends upon the correlation of in vitro with in vivo activity. This can be accomplished more easily with non-peptide ligands because they generally can penetrate the blood–brain barrier and therefore can be administered peripherally in vivo. Also, they are less subject to metabolism than are peptides.

In addition to their uses as pharmacological tools, selective, non-peptide opioid antagonists may have potential clinical applications in the treatment of a variety of disorders where endogenous opioids play a modulatory role. These include food intake, shock, constipation, immune function, behavior, CNS injury, and alcoholism.[13]

The Message–Address Model in the Design of Selective Non-Peptide Opioid Antagonists

The rationale for the design of such compounds was based on the message–address model which was employed by Schwyzer[14] to analyze structure–activity relationships of ACTH and related peptide hormones. Accordingly, peptide hormones are termed "sychnologic" if their information content is organized so that the "message" and

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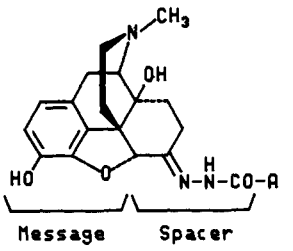

A (address)	Selectivity
Phe-Leu-OH	delta
Phe-Leu-Arg-Arg-Ile-OMe	kappa

Figure 2. Hybrid structures consisting of an opiate (message) and a peptide (address).

"address" components are proximal to one another in the peptide chain. The message component is required for triggering signal transduction at the receptor site; the address confers additional binding affinity and is not essential for the transduction process.

A cartoon to illustrate the concept, as applied to a family of receptor types (Figure 1), depicts each receptor type as having two subsites. These are (1) a message subsite which is similar or invariant for all of the receptor types and (2) an address subsite that is unique for each receptor type.

It was pointed out by Chavkin and Goldstein[15] that the endogenous opioid peptides conform to the message–address model; i.e., they contain a constant tetrapeptide sequence, Tyr-Gly-Gly-Phe, which can be viewed as the message, and a variable sequence which may serve as an address to confer selectivity for a receptor type.

A modified interpretation of the message–address model, as applied to opioid peptides, is that the Tyr^1 residue 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 to connect the message and adress elements. This is consistent with the well-known structure–activity relationships of non-peptide opioid ligands (e.g., morphine) that contain only one aromatic ring which presumably mimics the Tyr^1 residue.

This model was tested by the attachment of the address segments of leucine–enkephalin and dynorphin to oxymorphone, which contains a non-peptide message component (Figure 2).[16] The binding data revealed that a typically μ-selective ligand such as oxymorphone was transformed to a δ-selective ligand simply by attachment of the "δ address" (Phe-Leu) of leucine–enkephalin through a spacer to the C-6 position of the opiate. Similarly, a κ-selective ligand was obtained by attachment of a segment of the "κ address" (Phe-Leu-Arg-Arg-Ile-OMe) that is common to the endogenous κ-selective agonist dynorphin A.

These studies suggested that feasibility of developing non-peptide, δ-selective opioid antagonists by the attachment of a non-peptide moiety to an opiate structure in order to mimic a key recognition element in the address. Although the message–address concept was proposed for endogenous agonists, it could serve as a useful model for the design of selective antagonists as well, if such ligands

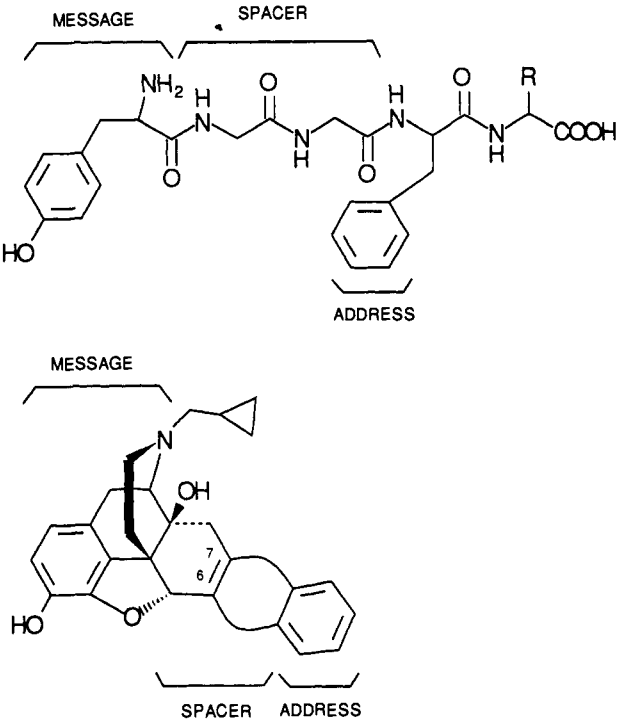

Figure 3. An approach to the design of a non-peptide δ-selective opioid antagonist based upon the message–address concept. The message and address components of the δ-selective peptide enkephalin (upper) are compared with those in an opiate (lower).

interact with the same message and address subsites of the receptor site.

The information content of the address is encoded by the amino acid sequence and its conformational constraints. The latter, which can be considered to be a hidden part of the address, determines the facility with which the address adapts conformationally in binding to an address subsite. The conformational mobility of the opioid peptides may contribute to their cross-recognition of opioid receptor types. This might occur by conformational adaption of the flexible peptide to the address subsite during the binding process, and it may provide a plausible explanation for the relatively low binding selectivity of the endogenous opioid peptides.[17]

Design Strategy for δ Opioid Antagonists

A strategy for the design of non-peptide, δ-selective antagonists was to employ a naltrexone-derived structure for the message moiety and a key element of the leucine–enkephalin δ address component.[18] 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 non-peptide to leucine–enkephalin is illustrated in Figure 3. The spacer should restrict the conformation of the benzene moiety (δ address mimic), and it was hoped that this would enhance δ selectivity by precluding conformational adaption of the address to subsites of non-δ opioid receptors.

The first target compound contained a pyrrole spacer because it was easily accessible from naltrexone in a single

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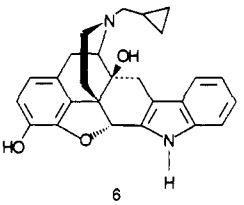
(18) Portoghese, P. S.; Sultana, M.; Takemori, A. E. Design of Peptidomimetic δ Opioid Receptor Antagonists Using the Message-Address Concept. *J. Med. Chem.* **1990**, *33*, 1714–1720.

Table I.[a] Comparison of the Antagonist Potency[b] and Affinity[c] of NTI (6) with Those of Other Antagonists

	antagonism					binding				
	K_e,[d] nM			K_e selectivity ratio		K_i, nM			K_i selectivity ratio	
antagonist	δ	μ	κ	μ/δ	κ/δ	δ	μ	κ	μ/δ	κ/δ
6 (NTI)	0.13	29	45	223	346	0.031	3.8	332	127	11066
8 (NTB)	0.27	27	48	100	178	0.013	19	152	1450	11700
7 (ICI174864)	69	>1667	>1250	>24	>18	35	>1000	>1000	>29	>29
5 (naltrexone)	32	1.0	5.5	0.04	0.17	36	0.8	20	0.02	0.6

[a] These data are taken from ref 18 and 22. [b] Tested on the guinea pig ileum preparation using agonists 1 (μ) and 2 (κ) and on the mouse vas deferens preparation using agonist 3 (δ). [c] Binding was conducted on guinea pig brain membranes. [d] K_e = [antagonist]/(IC_{50} ratio − 1).

synthetic step via the Fischer indole synthesis. This permitted quick access to the target compound in order to test the model. The target compound, naltrindole (6,



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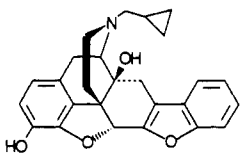
NTI), was the first reported[19,20] non-peptide δ opioid receptor antagonist. The in vitro δ antagonist potency is about 500 times greater than the δ-selective enkephalin analogue 7 [(allyl)$_2$Tyr-Aib-Aib-Phe-Leu-OH,[21] ICI174864]. In terms of binding, NTI has over a 1000-fold greater affinity than 7 (Table I).[18] The profound effect of the address moiety in NTI is also demonstrated by its 240-fold greater δ antagonist potency over its precursor, naltrexone (5).

The high antagonist potency and binding selectivity of NTI 6 are related to its greatly increased affinity for δ sites and to decreased affinity for non-δ opioid sites. This suggested that the benzene moiety of the indole system of NTI confers selectivity by binding to a part of the δ address subsite while hindering binding to other opioid receptor types.

Structure–Activity Relationship Studies

It is noteworthy that an NTI analogue 8 (NTB) which contains an isosteric spacer to hold the benzene moiety in the same orientation as NTI was also δ-selective, but with



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somewhat lower antagonist potency (Table I).[22] This benzofuran compound 8, however, possessed greater affinity for δ sites relative to that of NTI, and it also possessed greater binding selectivity. A possible reason for the lack of correlation between δ antagonist potency and binding may be related to the possible presence of δ receptor subtypes.[23]

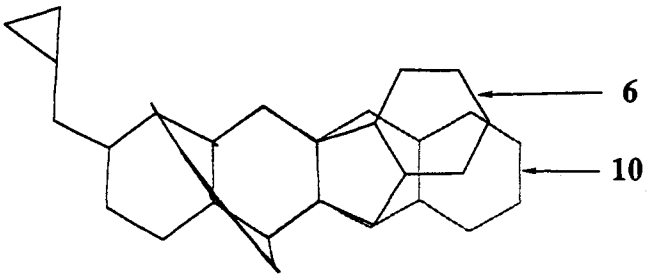
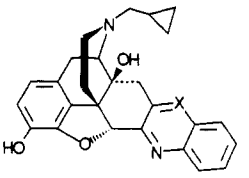


Figure 4. Superposition of NTI (6) with its quinoxaline analogue (10).

Analogues that contain quinoline (9) or quinoxaline (10) ring systems replacing the indole of NTI were less potent δ antagonists and had lower affinity for δ sites than NTI or NTB.[22] This may be due to the geometry of the spacers

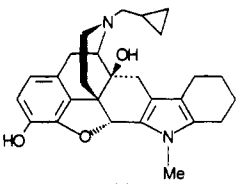


9 X = CH

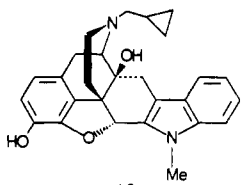
10 X = N

as they are six-membered rather than five-membered rings. As illustrated by the superposed structures (Figure 4), this orients the benzene moiety differently, thereby leading to lower affinity at the δ site and increased affinity at other sites.

Since it has been reported[24] that enkephalin analogues that contain a hexahydro-Phe[4] residue are less potent as opioid antagonists, we synthesized the tetrahydroindole analogue 11 to determine whether a similar relationship



11



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(19) Portoghese, P. S.; Sultana, M.; Nagase, H.; Takemori, A. E. Application of the Message–Address Concept in the Design of Highly Potent and Selective Non-Peptide δ Opioid Receptor Antagonists. *J. Med. Chem.* **1988**, *31*, 281–282.

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(22) Portoghese, P. S.; Nagase, H.; MaloneyHuss, K. E.; Lin, C.-E.; Takemori, A. E. Role of Spacer and Address Components in Peptidomimetic δ Opioid Receptor Antagonists Related to Naltrindole (NTI). *J. Med. Chem.* **1991**, *34*, 1715–1720.

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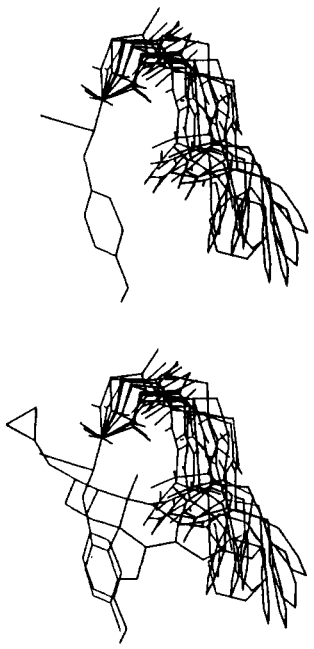

Figure 5. The conformations (at 0.5-ps intervals) of leucine–enkephalin derived from molecular dynamics simulations (300 K) during a 5-ps period in which the tyramine moiety of Tyr[1] has been fixed in a conformation identical with that of NTI (**6**) (upper illustration). Superposition of the tyramine moiety of **6** with that of enkephalin (lower) illustrates that there is overlap of conformational space occupied by the Phe[4] phenyl group and the indolic benzene moiety.

exists with NTI-related compounds.[22] The opioid antagonist profile of **11** was compared with that of *N*-methyl-NTI (**12**), which is a potent δ antagonist. The finding that **12** was 9-fold more potent than **11** is consistent with the role of the benzene moiety as a mimic of the phenyl group of Phe[4] in enkephalin.

It is noteworthy that **11** was apparently as δ-selective as its indole counterpart **12** despite its lower antagonist potency.[22] This illustrates an important point concerning the design of selective ligands; namely, that a concomitant proportional decline in the potency at all three receptor types can afford a highly selective ligand.

Conformational Relationship between NTI and Enkephalin

Molecular dynamics simulations were consistent with the idea that the Phe[4] of enkephalin and the indolic benzene moiety of NTI both bind to a common δ address subsite. The simulations of leucine enkephalin were carried out with the tyramine moiety of Tyr[1] immobilized in a conformation identical with that in the opiate structure; the remainder of the peptide was unrestrained (Figure 5).[22] This approach was taken in an effort to simulate a postulated zipper-type mechanism for binding of the peptide to the δ site. The zipper[25] model was employed because it offered a more reasonable alternative for the receptor binding of flexible peptides than a lock-and-key mechanism. This is because the conformational energy differences for amino acid residues generally are small. Thus, leucine–enkaphalin was envisaged to undergo nucleation of Try[1] at the message subsite of the δ recognition site followed by binding of the Phe[4] residue with a δ address subsite. The initial binding of the Tyr[1] residue was considered a reasonable assumption in view of the critical requirement of a protonated basic nitrogen in enkephalin and the fact that counterions are capable of attraction over greater distances than other types of interactions. The stepwise binding was envisaged to be accompanied by sequential conformational changes of the enkephalin residues leading to the fully bound ligand. Presumably, mutual conformational changes of the recognition site also occur during this process.

The results of these simulations showed that the conformational space occupied by the phenyl group of Phe[4] was restricted to the region of the indolic benzene moiety of NTI (Figure 5). The stability of the bent leucine–enkephalin backbone is consistent with the reported[26] conformational studies of δ-selective enkephalins and related peptides. This conformation may permit binding of the indolic benzene moiety to a locus of the δ address subsite that binds Phe[4] of enkephalin. However, it is unlikely that the Phe[4] phenyl group would conformationally adapt to an orientation identical with that of the indolic benzene moiety because complete superposition of both rings was not observed during the 300 K simulation. One possibility in this regard is that NTI stabilizes the δ receptor in an antagonist conformational state that is different from that in the agonist state. This could be a manifestation of different conformational requirements for agonists and antagonists.

Is There a Well-Defined Relationship between Selectivity and Affinity?

The fact that both NTI **6** and **7** (ICI174864) are highly δ-selective but differ dramatically in potency deserves comment, since opioid receptor type selectivity has been employed, on occasion, as a criterion for fit at a target receptor. There are basically two different ways in which enhanced selectivity may arise upon molecular modification. First, the modification may afford very large decreases in affinity for sites other than the target site, as is the case with the enkephalin analogue **7**. Alternately, the modification may lead to greatly enhanced affinity for the target site, with smaller affinity changes for other sites, as exemplified by NTI **6**. These examples clearly illustrate that there is no relationship between selectivity and affinity for the target site.

Ligands that fall into the first category should not be employed as models to study the topography of the traget site because their selectivity is derived from unfavorable interactions with other sites. On the other hand, compounds that fit into the second category may be better suited for modeling the target site, as it is more likely that the high selectivity in this case reflects specific interactions that enhance molecular recognition.

Summary and Conclusion

Approaches to the design of peptidomimetic ligands are currently of great interest because of the discovery of an

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increasing number of endogenous peptides that modulate physiological processes. The inherent lability of peptides and their poor oral absorption have made peptidomimetics attractive targets for drug development.

In this presentation I have discussed the design of a novel series of δ-selective opioid antagonists based on the message–address concept. The opioid peptides can be viewed to contain two elements: an essential message component that is recognized by the receptor subsite responsible for the signal transduction process and an address element that is recognized by a subsite that is unique to a single receptor type and functions to enhance binding to the site. Since the tyramine moiety in opiate structures is known to be important for activity, an identical element in Tyr[1] of the opioid peptides can be viewed as the message. A key moiety of the δ address was considered to be the phenyl group of Phe[4]. Combining the universal opioid antagonist naltrexone (5) with a strategically located address mimic afforded naltrindole (6, NTI), the first nonpeptide δ opioid receptor antagonist.

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Articles

Dexamethasone 21-(β-Isothiocyanatoethyl) Thioether: A New Affinity Label for Glucocorticoid Receptors

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The C-21 methanesulfonate ester of the synthetic glucocorticoid dexamethasone (Dex) is an efficient electrophilic affinity label of glucocorticoid receptors and exhibits irreversible antiglucocorticoid activity. In an effort to obtain other affinity labeling steroids with differing biological activities, several new derivatives of Dex were prepared which contained a reactive electrophilic substituent at various distances from the C-21 position. All compounds displayed relatively low affinity for rat glucocorticoid receptors (≤8% of that of Dex) in a cell-free competition assay. Nevertheless, one compound, dexamethasone 21-(β-isothiocyanatoethyl) thioether (Dex-NCS), appeared to be an affinity label by virtue of its ability to block the cell-free exchange binding of [^{3}H]Dex. [^{3}H]Dex-NCS was thus synthesized and reacted with cell-free receptors to give, after analysis on denaturing SDS-polyacrylamide gels, only one specifically labeled species at 98 kDa, which is the molecular weight of authentic rat glucocorticoid receptor. These data directly establish Dex-NCS as a new affinity label for glucocorticoid receptors. Data on the reactivity of Dex-NCS and the stability of [^{3}H]Dex-NCS-labeled receptors suggest that a cysteine SH group has been labeled.

Introduction

Affinity labeling of ligand-binding macromolecules gives covalent complexes which have numerous applications and can be studied under a greatly expanded variety of conditions. In the case of steroid receptor proteins, affinity labeling has been used to directly identify on denaturing SDS-polyacrylamide gels the native, mutant, and proteolyzed forms of receptor in various states of biological activity, purification, and chemical modification.[1-8] The covalent binding of the affinity label to the receptor protein is preserved during virtually all manipulations and facilitates the identification of molecules associated with the receptor (ref 7 and references therein). A classical use of affinity labels is to identify the amino acids involved in steroid binding.[8-12] A specialized use is to obtain irreversible agonists and antagonists.[13-17]

Despite the numerous applications of affinity labels for steroid receptors, the number of practical affinity labels is quite small.[18] This is not due to a paucity of methods for affinity labeling (for review, see ref 19). Rather, the discovery of new affinity labels has been hindered by the fact that most suitably modified ligands have such reduced

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