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Types of Opioid Receptors: Relation to Antinociception

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Types of opioid receptors: relation to antinociception

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The endogenous opioid peptides are derived from three large precursors. Pro-opiocortin and proenkephalin yield [Met]enkephalin, carboxy-extended [Met]enkephalins and [Leu]enkephalin. The fragments of prodynorphin are all carboxy-extended [Leu]-enkephalins. Three approaches are of importance for an analysis of the physiological functions of the different endogenous opioid peptides. First, since these peptides interact with more than one of the μ-, δ- and κ-binding sites and thus with their receptors, it is necessary to synthesize peptides or non-peptides, which bind to only one of the sites. As far as narcotic analgesics are concerned, morphine fulfils these conditions since it interacts almost exclusively with the μ-receptor. Secondly, antagonists are required that are selective for only one of the opioid receptors, even when used in high concentrations. Finally, it is important to find circumscribed areas in the nervous system that possess only one type of opioid receptor. It is now known that in the rabbit cerebellum the opioid receptors are almost exclusively of the μ-type whereas in the guinea-pig cerebellum they are almost exclusively of the κ-type.

1. Introduction

In a discussion on nociception and pain two fundamental problems arise: endogenous opioid ligands or opioid drugs bind to one or more sites of a complex macromolecule or receptor. Such an interaction may or may not lead to a biological response of the effector, initiated by activation of the binding site. An agonist compound may lead either to excitatory or inhibitory activity. An antagonist compound is characterized by its ability to block the excitatory or inhibitory responses of the effector. These considerations indicate that investigations of the binding of opioid peptides *per se* cannot decide whether endogenous or non-endogenous compounds act as agonists or antagonists at the receptor. A decision on this point depends on the response of the effector, which may be tested either *in vivo* in animals or humans, or *in vitro* on excised tissues. Assays of opioid compounds have led to useful results when preparations of different isolated tissues from different species are used. Thus, the myenteric plexus-longitudinal muscle preparation of the guinea-pig is affected by μ- and κ-ligands, the mouse vas deferens by μ-, δ- and κ-ligands, the rabbit vas deferens only by κ-ligands and the rat vas deferens probably only by μ-ligands.

The data available in the literature are by now so voluminous that it is necessary to consider mainly the more recent developments of particular importance in our understanding of the interaction of endogenous opioids with μ-, δ- and κ-receptors. In this context, particular use will be made of these endogenous peptides and analogues that are highly selective of only one of the three μ-, δ- and κ-receptors, either as agonists or antagonists. Further useful information is to be found in some recent reviews (Goldstein & Cox 1978; Simon & Hiller 1978; Kosterlitz & McKnight 1981; Robson *et al.* 1983; Paterson *et al.* 1984).

2. Affinities of some fragments of opioid precursors for the μ-, δ- and κ-binding sites

The three endogenous opioid precursors are pro-opiocortin, proenkephalin and prodynorphin. It is not proposed to enumerate all possible amino acid sequences, which could be active opioid fragments of the precursors; a selection has been made of those that appear to be of particular interest for our understanding of the action of endogenous and non-endogenous opioid peptides and also of some non-peptide opioids.

A presentation of the data has been chosen, which it is hoped will clarify some important points. The inhibitory binding constants of the opioids are given by $K_i = IC_{50}/(1+[L]/K_D)$, where [L] is the concentration of the labelled ligand and K_D its equilibrium dissociation constant (Cheng & Prusoff 1973). The binding affinity constant $(K_i, \text{nM})^{-1}$ is the reciprocal of the inhibition constant. Finally, to give an indication of the relative binding affinities at the μ-, δ-, and κ-sites the following equation is used: K_i^{-1} for μ, δ or κ/((K_i^{-1} for μ) + (K_i^{-1} for δ) + (K_i^{-1} for κ)).

(a) *Binding characteristics of fragments of proenkephalin and of* β*-endorphin*

The fragments of proenkephalin (Noda *et al.* 1982; Gubler *et al.* 1982) and β-endorphin, a fragment of pro-opiocortin (Nakanishi *et al.* 1979) vary in their relative affinities for the μ-, δ- and κ-binding sites (table 1). [Met]enkephalins extended at the C-terminus have affinities of varying degrees for the μ- or δ-binding site (table 1). Thus, β-endorphin, [Met]enkephalyl-Arg-Phe and [Met]enkephalyl-Arg-Arg-Val-NH_2 have affinities which are greater for the μ-site than for the δ-site, whereas [Met]enkephalyl-Arg-Gly-Leu and particularly [Met]enkephalin have higher affinities for the δ-binding site. As far as their relative affinities are concerned, β-endorphin and [Met]enkephalyl-Arg-Gly-Leu have values that are almost equal for μ- and δ-binding sites with only negligible affinity for the κ-binding site. Their absolute affinities for the μ-binding site vary considerably, ranging from 0.26 for [Met]enkephalyl-Arg-Phe to 8.7 for [Met]enkephalyl-Arg-Arg-Val-NH_2 (metorphamide (Weber *et al.* 1983), adrenorphin (Matsuo *et al.* 1983)). The latter member of this group has a significant relative affinity for the κ-binding site with only a negligible affinity for the δ-binding site.

Table 1. Binding affinities and relative binding affinities of [Met]enkephalin extended at the C-terminus at the μ-, δ- and κ-sites in homogenates of guinea-pig brain at 0 °C

	affinity		relative affinity		
	μ-site	δ-site	μ-site	δ-site	κ-site
β-endorphin	0.49	—	0.53	0.45	0.02
[Met]enkephalyl-Arg-Phe	0.26	—	0.70	0.27	0.03
[Met]enkephalyl-Arg-Arg-Val-NH_2	8.7	—	0.66	0.03	0.31
[Met]enkephalyl-Arg-Gly-Leu	—	0.21	0.41	0.56	0.03
[Met]enkephalin	—	1.10	0.09	0.91	0

The terms of affinity and relative affinity are defined in §2.

It should be noted that there are three endogenous opioid peptides with preferential affinity for the μ-binding site (table 1). However, it may be significant that the only compound that binds almost exclusively at the μ-site is of plant origin, namely morphine. To obtain peptides

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with a binding pattern similar to that of morphine, man has had to take recourse to synthesis in the laboratory. In this respect, one of the peptide analogues with satisfactory binding properties is [D-Ala^2, MePhe^4, Gly-ol^5]enkephalin since its affinity and relative affinities are very similar to those of morphine (table 3). However, it is not possible at present to compare its antinociceptive or analgesic potencies *in vivo* with that of morphine because there are significant differences in their abilities to pass through the blood–brain barrier.

We have an important problem that requires solution. There is little doubt that the antinociceptive effect of morphine in different animal tests and, more importantly, its analgesic effects in man are due to its interaction with μ-opioid receptors. Further pharmacological investigations are required to decide whether activation of δ- and κ-receptors can lead to clinical analgesia similar to that caused by the administration of morphine. In such an analysis we shall need compounds that act only on one of the three receptor types; ideally, they should be pure agonists or pure antagonists.

(*b*) *Binding characteristics of fragments of prodynorphin*

The fragments of prodynorphin (Kakidani *et al.* 1982) differ from those of pro-opiocortin and proenkephalin in that they have a high affinity for the κ-binding site (table 2). [Leu]enkephalins extended at the C-terminus, e.g. the octapeptide [Leu]enkephalyl-Arg-Arg-Ile, dynorphin A (1–8), the heptadecapeptide dynorphin A, the tridecapeptide dynorphin B and the decapeptide α-neo-endorphin have affinities greater for the κ-site than for the μ- or δ-sites; in contrast, [Leu]enkephalin has a greater affinity at the δ-site. With regard to their relative affinities, dynorphin A and dynorphin B have a very high relative affinity for the κ-binding site while dynorphin A (1–8) and α-neo-endorphin have a somewhat lower relative affinity for the κ-binding site and some increases in the relative affinities of the μ- or δ-binding sites. Their absolute affinities vary considerably, ranging from 0.75 for dynorphin A (1–8) to 8.5 and 8.7 for dynorphins B and A, respectively.

Table 2. Binding affinities and relative binding affinities of [Leu]enkephalin extended at the C-terminus at the μ-, δ- and κ-sites in homogenates of guinea-pig brain at 0 °C

	affinity		relative affinity		
	δ-site	κ-site	μ-site	δ-site	κ-site
[Leu]enkephalyl-Arg-Arg-Ile	—	0.75	0.22	0.16	0.62
[Leu]enkephalyl-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp-Asp-Asn-Gln (dynorphin A)	—	8.7	0.13	0.04	0.83
[Leu]enkephalyl-Arg-Arg-Gln-Phe-Lys-Val-Val-Thr (dynorphin B)	—	8.5	0.14	0.03	0.83
[Leu]enkephalyl-Arg-Lys-Tyr-Pro-Lys (α-neo-endorphin)	—	5.1	0.10	0.23	0.67
[Leu]enkephalin	0.85	—	0.06	0.94	0

The terms of affinity and relative affinity are defined in §2.

It is of interest that, in both the proenkephalin and prodynorphin series, [Met]enkephalin and [Leu]enkephalin are the only endogenous opioid peptides that have a high degree of selectivity for the δ-binding site. The possible significance of this observation is not yet understood.

The possible antinociceptive effects of dynorphin A (1–8), dynorphin A (1–13), dynorphin

A and dynorphin B were tested by the increase of latency in the tail flick response to radiant heat in rats (Han & Xie 1982; Przewlocki *et al.* 1983; Han *et al.* 1984). The dynorphins tested increased the latency of the tail flick and it was concluded this effect was due to interaction with κ-opioid receptors.

There are some interesting observations that may require further investigation (Przewlocki *et al.* 1983). The effect of dynorphin A (1–8) was of a relatively short duration and it was readily antagonized by pretreatment with 1 mg kg^{-1} naloxone. The effect of dynorphin A (1–13) lasted much longer; pretreatment with 10 mg kg^{-1} of naloxone attenuated the effect but 1 mg kg^{-1} had no effect. In an earlier investigation (Han & Xie 1982), intrathecal injection of dynorphin A caused a long-lasting increase of latency of the tail flick (up to 30 h). When 10 μg of dynorphin was injected through the intrathecal cannula together with 8 μg of naloxone, there was a partial reversal of the effect.

The findings with dynorphin B are of particular interest (Han *et al.* 1984). The IC_{50} of naloxone (mg kg^{-1}) was 0.7 for morphine and 0.3 for the highly selective μ-ligand [N-MePhe^3, D-Pro^4]morphiceptin, 0.8 for the partially selective δ-ligand [D-Ala^2, D-Leu^5]enkephalin, nine to ten for the selective κ-ligands dynorphin A and B. Since the concentrations of (−)-naloxone required to antagonize the κ-effect of dynorphin A and dynorphin B are rather high it would be of interest to test the effect of (+)-naloxone, and possibly also other antagonists. These controls would seem to be of importance since the non-opioid analogue [des-Tyr^1] dynorphin A (1–13) also increases the tail flick latency and produces paralysis as does dynorphin A (1–13) but not dynorphin A (1–8) (Przewlocki *et al.* 1983).

3. Selective non-endogenous opioid agonists and antagonists

To understand the functions of the μ-, δ- and κ-receptors three requirements are essential. First, the ligand must be highly selective for one of the receptors, secondly, the antagonists must be similarly selective and, thirdly, a small area of nervous tissue containing only one of the three binding sites may be used successfully as shown in the next section.

(*a*) *Binding affinities of selective non-endogenous opioid agonists*

As already stated, the endogenous opioid ligands interact with more than one of the μ-, δ- and κ-receptors. Many attempts have been made to synthesize peptides and non-peptides with high selectivity for one binding site only. An incomplete list is given in table 3 that includes the only alkaloid μ-agonist, morphine.

The first group with a high relative affinity at the μ-binding site consists of morphine and [D-Ala^2, MePhe^4, Gly-ol^5]enkephalin (Kosterlitz & Paterson 1981). They are potent in the binding assays and their interactions with the δ- and κ-sites are low.

The second group has two peptide analogues that were designed to improve the selectivity of [D-Ala^2, D-Leu^5]enkephalin at the δ-binding site: [D-Ser^2, L-Leu^5]enkephalyl-Thr (David *et al.* 1982) and [D-Pen^2, D-Pen^5]enkephalin (Mosberg *et al.* 1983). The second compound is more selective at the δ-binding site than the first but has a somewhat lower affinity.

The last group has compounds suitable for assaying selectivity for binding at the κ-site. None of them fulfils all the requirements enumerated in the first paragraph of this section. Ethylketazocine and bremazocine have also high affinities for the μ-site or for the μ- and δ-sites. Tifluadom and particularly U-50,488H are more selective for binding at the κ-site but their

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Table 3. Binding affinities and relative binding affinities of non-endogenous opioid agonists that are selective for the μ-, δ- or κ-sites in homogenates of guinea-pig brain at 25 °C

	affinity			relative affinity		
	μ-site	δ-site	κ-site	μ-site	δ-site	κ-site
normorphine	0.25	—	—	0.96	0.01	0.03
morphine	0.56	—	—	0.97	0.02	0.01
[D-Ala², MePhe⁴, Gly-ol⁵]enkephalin	0.54	—	—	0.99	0.01	0
[D-Ser², L-Leu⁵]enkephalyl-Thr	—	0.56	—	0.04	0.96	0
[D-Pen², D-Pen⁵]enkephalin	—	0.37	—	0.004	0.996	0
(−)-bremazocine	-	—	2.44	0.30	0.25	0.45
(−)-ethylketazocine	-	—	1.92	0.32	0.06	0.62
tifluadom	—	—	0.25	0.15	0.01	0.84
dynorphin A (1–9)	—	—	4.76	0.05	0.06	0.89
U-50,488H	—	—	0.13	0.01	0	0.99

The terms of affinity and relative affinity are defined in §2. Dynorphin A (1–9) was assayed at 0 °C. U-50,488H is trans-3,4-dichloro-*N*-methyl-*N*-(2-pyrrolidinyl)cyclohexyl)-benzeneacetamine (Piercey *et al.* 1982; Gillan *et al.* 1983).

affinities are low. Dynorphin A (1–9) is potent and highly selective for the κ-site; since it is readily degraded by peptidases, it is difficult to use in binding assays. At present, the most suitable assay compounds are [³H]bremazocine or [³H]ethylketazocine, in the presence of unlabelled [D-Ala², D-Leu⁵]enkephalin and [D-Ala², MePhe⁴, Gly-ol⁵]enkephalin to suppress δ- and μ-binding.

(*c*) *Binding affinities of antagonists of varying selectivity*

Only three representative antagonists are given in table 4. Naloxone has a high relative affinity at the μ-binding site; if the concentrations are raised it acts also as an antagonist at the κ-site and possibly at the δ-site. For this reason, and because of the possibility of non-opioid effects, it is advisable to exclude the non-specific effects by the use of (+)-naloxone when very high concentrations are used. ICI 174864 is a useful highly selective δ-antagonist (Cotton *et al.* 1984). However, its very low potency may cause difficulties when used *in vivo*. Mr 2266 is a potent κ-antagonist but its cross-reactivity to the μ-binding makes it insufficiently selective.

Table 4. Binding affinities and relative binding affinities of non-endogenous opioid antagonists at the μ-, δ- or κ-sites in homogenates of guinea-pig brain at 25 °C

	affinity			relative affinity		
	μ-site	δ-site	κ-site	μ-site	δ-site	κ-site
naloxone	0.56	—	—	0.85	0.06	0.09
ICI 174864	—	0.0052	—	0.01	0.99	0
Mr 2266	—	—	1.45	0.31	0.07	0.62

The terms of affinity and relative affinity are defined in §2. ICI 174864 is *N*,*N*-diallyl-Tyr-Aib-Aib-Phe-Leu-OH (Aib = α-aminoisobutyric acid) (Cotton *et al.* 1984; Corbett *et al.* 1984). Mr 2266 is as free base (−)-α-5,9-diethyl-2-(3-furylmethyl)-2′-hydroxy-6,7-benzomorphan (H. Merz, Boehringer, Ingelheim).

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4. Binding affinities in tissues with only one type of opioid receptor

In homogenates of the guinea-pig cerebellum, the binding of [^{3}H](−)-bremazocine is almost solely due to the κ-sites (Robson *et al.* 1984). This has been shown by saturation curves and Scatchard plots when the effect of addition of the unlabelled μ-ligand, [D-Ala^2, MePhe^4, Gly-ol^5]enkephalin (100 nM) and the unlabelled δ-ligand, [D-Ala^2, D-Leu^5]enkephalin (100 nM) was found to be very small (figure 1). Furthermore, the K_i value for inhibition of [^{3}H](−)-bremazocine (0.1–0.2 nM) binding by [D-Ala^2, MePhe^4, Gly-ol^5]enkephalin or by [D-Ala^2, D-Leu^5]enkephalin was greater than 1000 nM, a concentration that caused only 24% and 13% inhibition, respectively.

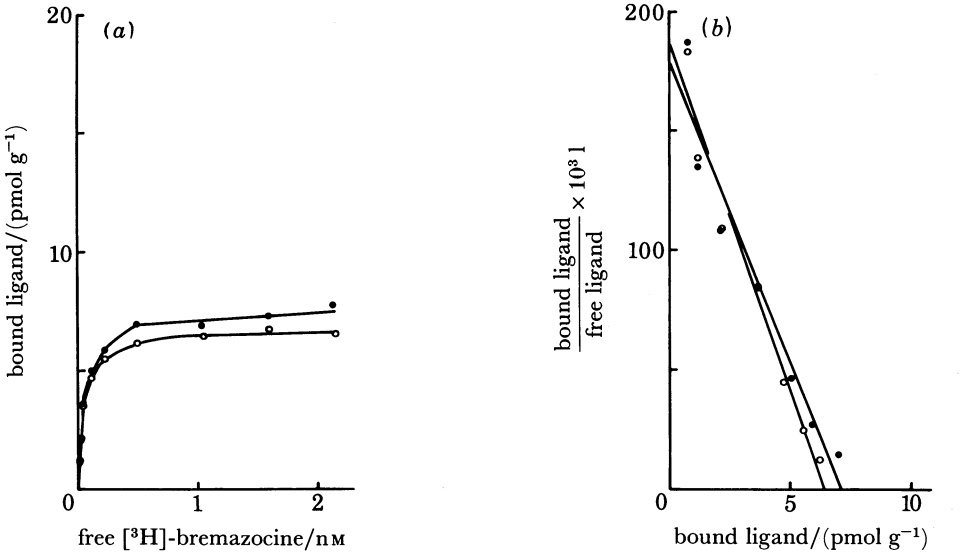

Figure 1. Homogenate of guinea-pig cerebellum. Saturation curve (*a*) and Scatchard plot (*b*) of the specific binding of [^{3}H](−)-bremazocine in the absence (●) and presence (○) of unlabelled [D-Ala^2, MePhe^4, Gly-ol^5]enkephalin and unlabelled [D-Ala^2, D-Leu^5]enkephalin at a ratio of 100 nM ligand to 0.1 nM [^{3}H](−)-bremazocine. Single result typical of three experiments. (With permission from Robson *et al.* (1984).)

There is considerable variation in the number of opioid binding sites in various species. For instance in the rabbit, the opioid binding sites in the cerebellum are mainly of the μ-type (Meunier *et al.* 1983) whereas there are no significant opioid binding sites in the cerebella of adult mice, hamsters or rats (Meunier & Zajac 1979).

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