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COMMENTARY

MULTIPLE OPIATE RECEPTORS IN PERIPHERAL TISSUE PREPARATIONS

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Major interest in the field of opiate research focuses on the issue of a heterogeneity of receptors for opioids. Although the molecular structure of the opioid receptor macromolecule remains unknown, two separable functions have to be postulated: (1) the ability of the receptor to recognize a specific drug and (2) the capacity to translate a received signal. Thus, during recent years both the recognition of an opioid as well as the pharmacologic response have been employed for the characterization of opiate receptors. However, one has to keep in mind that description of recognition sites, which in general relates to binding studies conducted with radioactively labelled compounds, is limited in its value as compared to the ability of the receptor both to recognize and to mediate a specific information received. In fact, the concept of multiple opiate receptors originates from careful observations of different pharmacological effects being elicited by morphine and structurally related compounds [1, 2]. More recently, following the discovery of the opioid peptides, this concept gained further attention as documented by numerous papers, putting emphasis on the differentiation of opiate recognition sites particularly in the central nervous system (CNS). These findings have been comprehensively reviewed by others [3–5]. Apparently, further distinctions between opiate binding sites can be achieved by their selective protection with specific opioids against alkylating inhibitors of binding [6, 7], with sodium ions or with the guanine nucleotide GTP [8]. Significant contributions to the concept of multiple opiate receptors come from isolated preparations. The classical preparation for investigation into the action of opioids is the electrically stimulated guinea-pig ileum (GPI). More recently, the mouse vas deferens (MVD) and later the rat vas deferens (RVD) received particular attention as they exhibit specific sensitivity to opioid peptides. Since the potency of a number of opioids does not vary in parallel between these tissues, a different receptor population was proposed to be inherent in each tissue.

The action of opioids in naive preparations

Striking variations were observed in the rank order of potencies of different opioids to inhibit electrically evoked twitches of the GPI and the MVD. Since the GPI proved highly sensitive to morphine and structurally related drugs, but relatively less sensitive to the enkephalins, Lord *et al.* [9] considered this preparation to contain predominantly μ-opiate receptors. In contrast, the MVD was classified to contain predominantly δ-receptors because of its high sensitivity to the enkephalins. However, a simultaneous existence of other receptor types in these tissues is indicated. A survey of opiate receptors, so far identified, is given in Table 1.

Another interesting preparation represents the RVD as judged by its response to opioids. Neither morphine nor the enkephalin-pentapeptides significantly affect electrically evoked twitches (at 10^{-4} M 10–20% inhibition). However, the long-chained peptide β-endorphin exhibits a similar potency as compared to that in the GPI and MVD. Detailed investigations revealed that an activation of opiate receptors in this preparation requires a certain minimal length of the β-endorphin sequence. As demonstrated in Fig. 1, the β-endorphin fragment 1–23 is of similar high potency as β-endorphin itself.

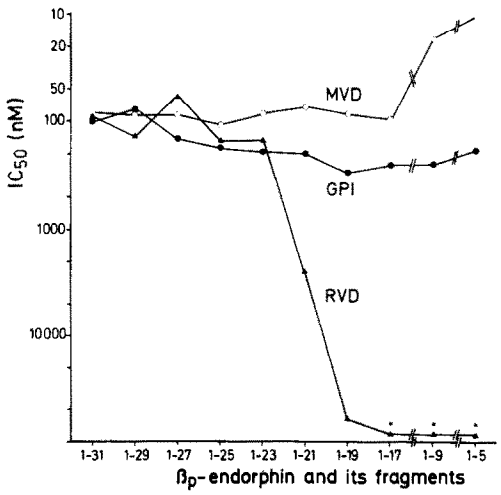

Fig. 1. Opiate activity of β-endorphin (sequence 1-31) and its fragments on the electrically stimulated mouse vas deferens (○, MVD), the longitudinal muscle-myenteric plexus preparation of the guinea-pig ileum (●, GPI) and the rat vas deferens (▲, RVD). Each point reflects the mean concentration (nM) of peptides required to cause half-maximal inhibition of twitch tension (IC_{50}). The standard errors of the mean ranged from 2 to 13% (N = 6–8) (from Schulz *et al.* [13]. * Less than 20% opiate-like inhibition at 8×10^{-5} M.

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Table 1. Identified opiate receptors

Type of receptor	First detected in	Characteristic ligand
μ	CNS, GPI	Morphine
δ	MVD	Enkephalin, [D-Ala^2,D-Leu^5]-enkephalin
ε	RVD	β-Endorphin
σ	CNS	SKF 10047*
κ	CNS, MVD	Ketocyclazocine, MR 2034†, MRZ‡, dynorphin$_{1-13}$§

* *N*-allylnormetazocine.
† 5,9-dimethyl-2′-hydroxy-2-tetrahydrofurfuryl-6,7-benzomorphan (Merz *et al.* [10]).
‡ (5,9-dimethyl,2″S)-5,9-dimethyl-2′-hydroxy-2(2-methoxy-propyl)-6,7-benzomorphan (Merz and Stockhaus [11]).
§ dynorphin$_{1-13}$ (Goldstein *et al.* [12]).

Shorter fragments dramatically lose opiate activity in the RVD, which contrasts the findings obtained with the GPI and the MVD. These unique properties of the RVD-receptors in its response to opioids caused Wüster *et al.* [14] to propose the term ε-opiate receptor.

The activity of opioids were also tested by others in different peripheral structures than those already mentioned. It appears that the rat colon, the ileum of the rabbit and the rat, the esophagus of the chicken or the intestinal mucosa are highly sensitive to enkephalins, suggesting a mediation of the opiate effect predominantly via δ-receptors.

The supposed existence of specific types of opiate receptors in distinct preparations initiated investigations into the specificity of a number of opioids, assuming that the GPI is representative for μ-receptors, the MVD for δ-receptors and the RVD for ε-receptors. Figure 2 reflects the preference (expressed in per cent) of a number of opioids to each of the respective receptors, as has been calculated on the basis of their potency on each preparation to half-maximally inhibit electrically induced twitches. β-Endorphin, which displays about an equal potency on each preparation, is accordingly located in the center of this arrangement. In contrast,

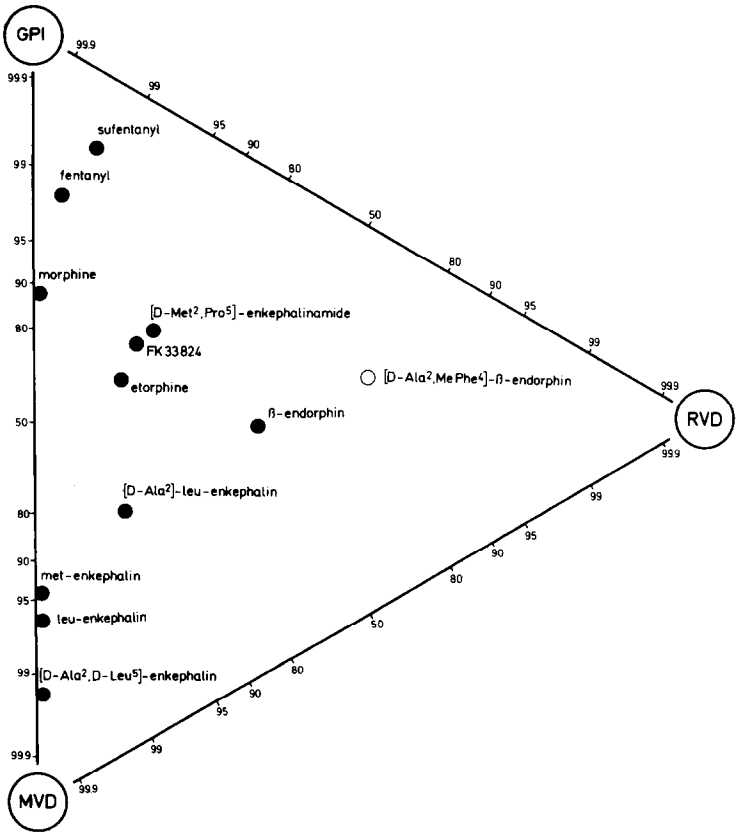

Fig. 2. Specificity of opioids towards the GPI (μ-receptor), the MVD (δ-receptor) and the RVD (ε-receptor). The position within this arrangement is determined by the relative potencies of each compound on the three assay systems to half-maximally inhibit electrically evoked twitches. To make more clear small differences between highly selective opioids, a graduation according to the probit scale was employed.

morphine and in particular the potent narcotic agonist sufentanyl (SUF) exhibit a 90 per cent, or even higher, preference to μ-receptors. However, there is presently no endogenous ligand known, which possesses a high preference to this class of receptors. On the other hand, methionine- and leucine-enkephalin both display more than 90 per cent preference for δ-receptors, while synthetic derivatives of the enkephalins, in general, show an increased selectivity for μ-receptors—best documented by the Sandoz compound FK 33824. One striking exception, however, is represented by [D-Ala^2,D-Leu^5]-enkephalin (DADL), since this derivative has increased preference for δ-receptors (99 per cent). With respect to the ε-receptor no agonist of high preference is presently known. [D-Ala^2-MePhe^4-β-endorphin with an assumed 70 per cent direction to the ε-receptor indicates that there may be a chance to synthesize drugs of higher selectivity for this kind of receptor. Such a drug is badly needed not only for the demonstration but also for the investigation of the functional role of ε-receptors outside the RVD.

This system of classification of opioid agonists brings to mind that specific drugs of high preference to μ- and δ-receptors, respectively, are presently available. However, an overlapping may occur as higher doses are employed.

Attempts to differentiate opiate receptors using narcotic antagonists have largely failed. Although μ-receptors are about 10-fold more sensitive to naloxone as compared to δ-receptors, a highly selective antagonist for only one type of opiate receptor—as is available, e.g. for the different types of adrenergic receptors—is presently missing. However, the significance of this issue has been realized and efforts may be successful to obtain such drugs as is shown by the synthesis of antagonists with some preference for the κ-receptors.

Although the MVD has, so far, been regarded a δ-receptor preparation, several indications point to the existence of additional types of opiate receptors. Therefore, a series of careful investigations was conducted in order to study the multiplicity of opiate receptors in the MVD. One approach to distinguish opiate receptors in the MVD is brought about by the monovalent cation potassium. In general, opioids lose activity to inhibit electrically evoked twitches as the concentration of potassium increases in the bathing fluid. Least sensitive to this effect of potassium were μ- and δ-opiate receptor agonists, displaying only a moderate loss when K^+ was elevated 3-fold. In contrast, dynorphin $_{1\text{-}13}$ (DYN) as well as the supposed κ-agonists exhibited at corresponding potassium concentrations a dramatic loss of potency (up to 8-fold). Obviously, potassium preferentially controls those receptors in the MVD mediating the action of DYN and κ-agonists.

The action of opioids in tolerant preparations

The concept of multiple opiate receptors does not define the character of the receptor, that is, whether multiple recognition sites are located at a single receptor macromolecule or whether distinct recognition sites are associated with distinct effector systems mediating a received signal. In an attempt to further investigate this problem, the phenomenon of tolerance development upon chronic opiate exposure was employed. If multiple opiate receptors do exist independently of each other, a selective tolerance development upon their chronic activation could be expected. An essential prerequisite for such studies is the availability of selectively acting agonists such as SUF (μ-receptors) and DADL (δ-receptors) (Fig. 2).

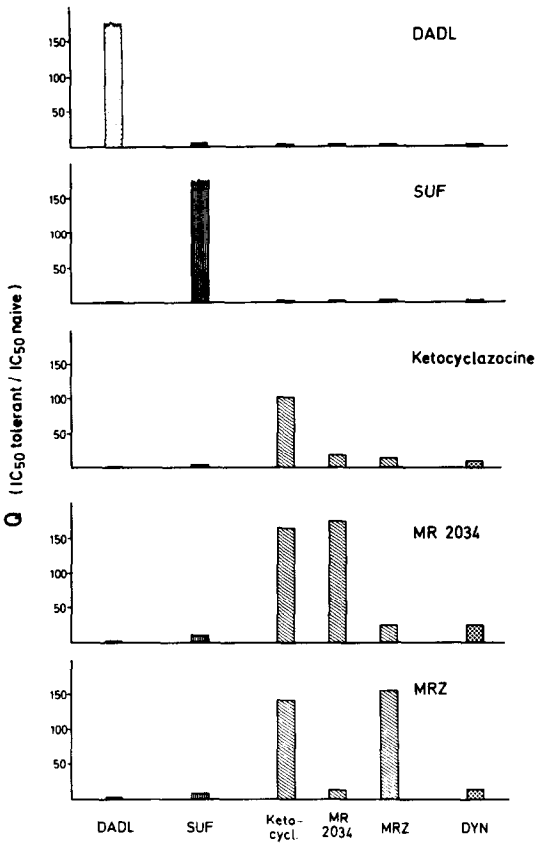

Fig. 3. Tolerance and cross-tolerance caused by different opiate receptor agonists in the mouse vas deferens. Mice were infused by means of osmotic minipumps (1 μl/hr) implanted subcutaneously for 6 days. Thereafter, the vasa deferentia were removed and set up *in vitro* for electrical stimulation to test the degree of tolerance to different agonists. For experimental conditions employed see Schulz *et al.* [15]. Columns reflect the degree of tolerance measured (Q = 1 indicates lack of tolerance). Vasa deferentia of mice infused with DADL and SUF, respectively, proved more than 160-fold tolerant to the respective opioid chronically applied. Abscissa: opioids under investigation. The opioid noted in each panel indicates the drug chronically given to the mice.

The MVD proved a unique preparation to study this hypothesis. Mice were chronically infused with a selective agonist, employing osmotic minipumps (ALZA Corp.). Their vasa deferentia were set up *in vitro* at the corresponding blood concentration in order to maintain tolerance after the tissues have been removed from the animals. These preparations were then subjected to tolerance development by different opioids (Fig. 3). Lack of tolerance is indicated by a quotient (Q) = 1, that is, the sensitivity

of a naive preparation to a certain drug is identical to that observed in a preparation chronically exposed to an agonist. Therefore, Q, increases as the degree of tolerance increases.

Figure 3 demonstrates tolerance, cross-tolerance and the apparent lack of cross-tolerance in such preparations. Apparently, preparations of DADL-infused mice display a high degree of tolerance to DADL and other δ-agonists [15] whilst the potency of SUF (μ-agonist), ketocyclazocine, MR 2034, MRZ (supposed κ-agonists) and DYN remains unchanged. By analogy, a selective tolerance development for SUF was observed in vasa deferentia of SUF-infused mice, whilst cross-tolerance to the other compounds tested was missing.

The issue of specific opiate receptors such as μ-, δ-, κ-receptors may be complicated by the phenomenon of the existence of subtypes of the particular receptor populations. This notion has been tested for the supposed κ-receptor agonists ketocyclazocine, MR 2034 and MRZ. Whereas the chronic treatment with either compound failed to induce tolerance on μ- and δ-receptors, an interesting pattern of cross-tolerance was exhibited by the three mentioned κ-agonists. Vasa deferentia of mice chronically exposed to ketocyclazocine displayed a high degree of tolerance to ketocyclazocine itself, but only a minor degree of cross-tolerance was observed for MR 2034 and MRZ. Chronic exposure to MR 2034 resulted in both a high degree of tolerance to the infused benzomorphan as well as a high degree of tolerance to ketocyclazocine. However, the potency of MRZ was only moderately affected in those preparations. On the other hand, tolerance induction to MRZ was associated with cross-tolerance to ketocyclazocine, but not to MR 2034. These findings clearly demonstrate a heterogeneity of receptor specificity even within a series of supposed κ-agonists.

Figure 3 also demonstrates the distinct properties of DYN as compared to the μ-, δ- and κ-agonists. Obviously, no interaction exists with μ- and δ-agonists, while some degree of cross-tolerance exists with the supposed κ-agonists. Thus, these data support the idea that DYN interacts with κ-opiate receptors and confirm reports which almost definitely rule out μ-agonistic properties of DYN in the MVD [16], although Chang *et al.* [3] proposed such a relationship for the CNS.

An identical approach for the differentiation of opiate receptors by means of their selective development of tolerance has also been employed to delineate opiate receptors in the CNS. In general, the selectivity of opioids such as SUF and DADL, which show almost no overlapping in the MVD, is much less pronounced in this tissue [17]. This phenomenon suggests that the binding sites of these receptors may be differentially organized in the CNS and the MVD.

The considerable different findings in the MVD and the CNS may give rise to speculations concerning adaptational mechanisms upon chronic exposure to opioids. The MVD is considered to contain nerve terminals only, that is, opiate receptors should be located exclusively at presynaptic sites. In contrast, opiate receptors in the CNS are supposedly found both at pre- as well as at postsynaptic sites. For the CNS dramatic withdrawal signs can be precipitated by the narcotic antagonist naloxone during the state of tolerance. This fact as well as the overwhelming findings that neither the number nor the affinity of opiate receptors change upon chronic exposure to opiate agonists, let assume that adaptational processes take place beyond the opiate receptor. In contrast, withdrawal signs have not been detected in the extremely tolerant MVD and this may suggest a different adaptational mechanism at structures with presynaptically located receptors.

Conclusions

The different potencies of different opioids in various peripheral tissues can best be explained by the concept of multiple opiate receptors. Since the classification of opiate receptors is rather arbitrary, as they are described by agonists, it is not surprising that compounds supposed to belong to the same pharmacological class (e.g. κ-receptor agonists) exhibit an individual profile of action. This is convincingly demonstrated for κ-agonists by their ability to bring about tolerance and/or cross-tolerance. On the basis of these findings one may even differentiate between subtypes of κ-receptors. Analogous, an extrapolation of these data would suggest subtypes for the other opiate receptors known. In any case, the data presented here are in support for an existence of κ-opiate receptors, originally proposed for the CNS. This conclusion rests upon the demonstration of selective induction of tolerance of κ-receptors in the MVD and is in conflict to the inability of binding studies with brain homogenate to demonstrate κ-receptors therein [18–20].

A further interesting phenomenon is the fact that derivatives of opioid peptides, such as the stable analogues of the enkephalins or of DYN, in general lose their direction towards their specific receptors and gain μ-receptor preference. Similarly, the ε-receptor requires for its activation a certain length of the amino acid chain of β-endorphin, and this biological activity is rapidly lost below a critical size. These phenomena—taken together—suggest that a rank order of specificity between opiate receptors may exist, of which the μ-receptors may be the least restrictive ones. In line with this interpretation would be findings with naloxone. Naloxone is a powerful antagonist at μ-receptors, but loses activity at δ- and κ-receptors. The fact that naloxone is about equally potent at μ- and ε-receptors does not invalidate this hypothesis, assuming that the blockade of a very specific receptor region by naloxone, which is required beside others to bring about the opiate-like effect of the long-chained β-endorphin, could completely eliminate the agonistic property of this peptide.

In summary, the presently available data strongly support the heterogeneity of ligand specificity of opiate receptors. However, whether this multiplicity is due to a different mode of interaction with a single receptor macromolecule carrying different binding sites or whether distinct receptor entities do in fact exist remains to be elucidated.

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