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Phil. Trans. R. Soc. Lond. B 1985 **308**, 299-310
doi: 10.1098/rstb.1985.0030

Phil. Trans. R. Soc. Lond. B **308**, 299–310 (1985)
Printed in Great Britain

Multiple opioid ligands and receptors in the control of nociception

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This paper summarizes the results of recent data characterizing the role of endogenous opioid peptides and opioid receptors in nociception. In addition, evidence is given that antinociception induced by intracerebroventricular injection of opioids into mouse brain is mediated by receptors resembling those mediating the inhibitory action of these substances on the rat vas deferens (putative ε-receptors). The endogenous ligands for these receptor are β-endorphin and the peptides deriving from pro-enkephalin A.

1. Introduction

In the course of recent years, our knowledge concerning the multiplicity of opioid systems has greatly expanded. It is now known that there are at least three families of endogenous opioid peptides, each derived from a distinct precursor, and each possessing a differential distribution in nervous tissues. In addition, there is substantial pharmacological evidence for the existence of multiple opioid receptors. This issue of the complexity of opioid systems raises several, as yet, unresolved questions: (i) Do different opioid peptide families have a particular preference for a certain class of opioid receptors? (ii) Which opioid peptides are involved in modulating nociception? (iii) Is there any opioid receptor specific for analgesia?

In this paper we will summarize recent information and offer certain novel results of pertinence to these questions.

2. Multiple opioid systems

(*a*) *Multiple opioid peptides*

Since the discovery of the enkephalins, numerous endogenous opioid peptides have been isolated which possess the amino acid sequence of either [Met]- or [Leu]enkephalin at their N-termini. By use of recombinant DNA techniques the sequence of the precursors for these peptides was deduced from the respective cloned cDNAs and three distinct opioid peptide families were distinguished (for review see Höllt 1983; Hughes 1983); pro-opiomelanocortin (POMC) is the precursor for β-endorphin and corticotropin; pro-enkephalin A contains four [Met]enkephalins, one [Leu]enkephalin, one heptapeptide ([Met]enkephalyl-Arg^{6}-Phe^{7}) and one octapeptide [Met]enkephalyl-Arg^{6}-Gly^{7}-Leu^{8}, each sequence separated by a pair of basic aminoacids. Since, however, the order of cleavage does not depend only on the dibasic aminoacids, several larger peptides are generated such as peptide F, peptide E and BAM- (= bovine adrenal medulla) peptides. Similarly, pro-enkephalin B (= pro-dynorphin) can be cleaved not only into three [Leu]enkephalins but also into larger opioid peptides (neo-endorphins, dynorphins, [Leu]morphin). In addition, two C-terminally amidated peptides originating from pro-enkephalin A have been recently isolated: the octapeptide

[Met]enkephalyl-Arg^6-Arg^7-Val^8-NH_2 (metorphamide (Weber *et al.* 1983); adrenorphin (Matsuo *et al.* 1983) and an amidated peptide comprising the first 26 aminoacids of bovine peptide F (amidorphin: Liebisch & Seizinger 1984)). All these peptides have been localized in pituitary, brain, gastro-intestinal tract and adrenal medulla by immunochemical techniques. These studies indicate, in general, a qualitative and quantitative differential distribution of the peptides of the three opioid families, although all three opioid systems can co-exist in the same cell (e.g. chromaffin cell of the human adrenal medulla).

(*b*) *Multiple opioid receptors*

There is considerable evidence for the existence of multiple opioid receptors. Martin *et al.* (1976) classified opioid receptors in terms of their effects in the chronic spinal dog as being morphine-like (μ), ketocyclacozine-like (κ) and *N*-allylnormetazocine-like (σ). Lord *et al.* (1977) have identified a further receptor in the mouse vas deferens: a preparation in which the enkephalins have a particularly high inhibitory potency; this receptor was designated to δ-receptor. The relatively high potency of β-endorphin in the rat vas deferens prompted the suggestion that its effect is mediated by a further type of opioid receptor, the ε-receptor (Wüster *et al.* 1979). With the exception of those of the ε-receptor, the binding sites of these receptors have been demonstrated by binding studies *in vitro* (for review see Paterson *et al.* 1983).

Recent experiments indicated that the σ-receptor is not a true opioid receptor, since the prototypic substance *N*-allylmetazocine interacts with specific binding sites for the non-opioid phencyclidine (Zukin & Zukin 1981).

Thus, there is agreement for the existence of four opioid receptor types: μ, κ, δ and ε. It has to be mentioned in this context that the ε-receptor is as yet poorly defined and not generally recognized to be a distinguishable receptor type. Similarly, the further subdivision of the μ-receptor into μ 1- and μ 2-receptors (Pasternak 1981) and of the κ-receptor into κ 1 and κ 2 (Gouarderes *et al.* 1982) has not, as yet, found general acceptance.

(*c*) *Receptor selectivities of the three opioid peptide families*

There are now an increasing number of reports attempting to allocate the different opioid peptide families to the different types of opioid receptors (Corbett *et al.* 1982; Paterson *et al.* 1983; Höllt *et al.* 1983; Quirion & Weiss 1984; James *et al.* 1984). From these studies it appears that:

(i) The δ-receptor does not have preferential affinity for one of the opioid peptide families. Pro-enkephalin A and pro-enkephalin B (= pro-dynorphin) give rise to [Met]enkephalins and/or [Leu]enkephalins which are prototype ligands for the δ receptor. In addition, β-endorphin the principal opioid product of POMC has also been found to possess significant affinity for the δ-receptor.

(ii) The μ-receptor has a preference for β-endorphin and several pro-enkephalin A derived peptides such as peptide E, BAM-22P, BAM-12P and metorphamide (Höllt *et al.* 1983; Weber *et al.* 1983). The pro-enkephalin B (= pro-dynorphin)-derived peptides (dynorphins, neo-endorphins) display some affinity for the μ-receptor which is, however, relatively low as compared to their high affinity for κ-receptors (Garzon *et al.* 1983).

(iii) The κ-receptors exhibit high avidity for all pro-enkephalin B (= pro-dynorphin) derived peptides (with the exception of [Leu]enkephalin). All peptides (dynorphins, neo-endorphins, [Leu]morphin) are active on the rabbit vas deferens preparation which contains κ opioid

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receptors exclusively (Oka *et al.* 1980; Corbett *et al.* 1982; Suda *et al.* 1983; Rezvani *et al.* 1983; Quirion & Weiss 1983). Substantial κ-activity also resides in some of the pro-enkephalin A-derived peptides, such as peptide E, BAM-22P, BAM-12P, metorphamide and the octapeptide [Met]enkephalyl-Arg^6-Gly^7-Leu^8 (Schulz *et al.* 1982; Garzon *et al.* 1983; Rezvani *et al.* 1983; Zajak, 1983; Quirion & Weiss 1983; Weber *et al.* 1983; Höllt *et al.* 1983). In contrast, β-endorphin and the enkephalins have no effect on the rabbit vas deferens which contains κ-receptors exclusively (Oka *et al.* 1980; Oka & Negishi 1982; Rezvani *et al.* 1983).

(iv) The ε-receptor was postulated to exist in view of the uniquely high potency of β-endorphin on the rat vas deferens (Wüster *et al.* 1979). Recent experiments have, however, indicated that in the presence of enzyme inhibitors the enkephalins also possess substantial activity on this preparation (McKnight *et al.* 1983; see also table 1). Interestingly, all the pro-enkephalin B (= pro-dynorphin)-fragments (with the exception of [Leu]enkephalin) were devoid of any inhibitory activity: even in the presence of enzyme inhibitors (see table 1; McKnight *et al.* 1982). Recently, we found that essentially all pro-enkephalin A-derived peptides tested (including the enkephalins) possess substantial agonist potency on the rat vas deferens (table 1).

TABLE 1. INHIBITORY POTENCY OF ENDOGENOUS OPIOID PEPTIDES ON PERIPHERAL BIOASSAYS AND ANTINOCICEPTIVE POTENCY IN THE MOUSE

opioid peptide	guinea-pig myenteric plexus IC_{50}/nM	p.s.	mouse *vas deferens* IC_{50}/nM	p.s.	rat *vas deferens* IC_{50}/μM	p.s.	rabbit *vas deferens* IC_{50}/μM	p.s.	antinociception ED_{50}/(nmol/mouse i.c.v.)
β-endorphin (human)	67.1±5.0	1	81.7±6.2	1	0.04±0.01	1	> 100	/	0.05 (0.03–0.08)
[Met]enkephalin	137.2±9.7	15	20.8±8.5	15	28.0±5.4	58	> 100	/	130.3 (87–195)
[Met]enkephalyl-Arg^6-Phe^7	133.1±21.4	13	15.5±2.6	13	20.1±4.2	27	58.9±13.5	7	49.6 (35–69)
[Met]enkephalyl-Arg^6-Gly^7-Leu^8	1140.2±330.0	36	549.7±68.4	40	35.3±6.6	43	64.1±14.1	7	105.7 (70–158)
BAM-12P	127.5±29.7	26	226.6±26.6	6	16.1±3.1	2	10.6±2.8	6	56.2 (35–87)
BAM-22P	0.6±0.2	1	41.7±5.6	3	1.2±0.3	2	9.9±2.1	2	1.5 (1–2)
peptide E	2.2±0.4	1	49.6±6.3	4	2.9±0.9	3	14.8±4.4	2	2.1 (1–3)
peptide F	293.6±49.7	2	270.3±15.5	4	3.0±1.2	2	> 30	/	3.0 (2–5)
[Leu]enkephalin	269.9±28.3	14	9.7±2.1	11	22.0±5.4	41	> 100	/	120.0 (80–179)
α-neoendorphin	18.5±4.3	14	50.0±3.6	5	> 100	/	3.0±0.8	8	> 100
β-neoendorphin	47.8±11.2	6	10.0±1.0	6	> 100	/	4.7±1.3	5	> 100
dynorphin 1–8	217.3±16.1	25	112.8±9.9	12	> 100	/	4.7±0.7	9	> 100
dynorphin A	0.3±0.1	1	0.9±0.2	1	> 100	/	0.3±0.1	1	> 100
dynorphin B (= rimorphin)	10.6±2.0	8	25.3±27.0	6	> 100	/	1.6±0.3	4	< 100
leumorphin	3.9±0.4	n.d.	39.5±6.5	n.d.	> 100	n.d.	0.5±0.2	1	> 100

(Concentrations of peptides that inhibit the muscle twitch by 50% (IC_{50}) were determined from logarithmic dose–response curves, as described previously (Sanchez-Blazquez *et al.* 1984). Values are geometric means±s.e.m. of 4–20 independent measurements. The IC_{50} values for the pro-enkephalin B (= pro-dynorphin)-derived peptides on the guinea-pig ileum and the mouse vas deferens are from Sanchez-Blazquez *et al.* (1984). All IC_{50} values on the rabbit vas deferens are from Rezvani *et al.* (1983). p.s. (= potency shift) represents the ratio of the potency in the presence to that in the absence of enzyme inhibitors. The enzyme inhibitors and their concentrations are the same as described recently (McKnight *et al.* 1983). /, The IC_{50} could not be determined even in the presence of enzyme inhibitors; n.d., not determined. The antinociceptive potencies (ED_{50}) are the doses in nanomoles of the peptides required to increase the threshold for the tail-flick latency in 50% of the mice after i.c.v. injection. Data are taken from Höllt *et al.* (1982, 1983). In addition, ED_{50} for [Met] and [Leu]enkephalin have been included, measured at their maximum effect 5 min after injection. Values are means±95% confidence intervals calculated from dose–response curves constructed from at least three doses, each dose given to eight to ten mice.)

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In conclusion, there appears to exist a certain preference of the three opioid peptide families for the four types of opioid receptors: β-endorphin, endproduct in the processing of POMC, shows affinity for μ-, ε- and δ- but not for κ-receptors.

Pro-enkephalin B (= pro-dynorphin) generates a variety of peptides with selectivity for κ- (dynorphins, neo-endorphins) and δ- [leu]enkephalin but not for μ- and ε-receptors.

Pro-enkephalin A gives rise to peptides with selectivities for all four types of opioid receptors: μ- (BAM-22P), ε- (peptide F), κ- (metorphamide) and δ- [Met] and [Leu]enkephalin.

3. Opioid receptors involved in analgesia

(*a*) *Supraspinal receptors*

Studies on the antinociceptive effects of intracerebrally (i.c.) administered opioid alkaloids and opioid peptides have indicated that the periaqueductal gray (PAG) and the rostral ventral medulla are major loci for analgesically effective opioids. Using specific antibodies for peptides derived from pro-enkephalin A [Met]enkephalyl-Arg^{6}-Gly^{7}-Leu^{8}) and pro-enkephalin B (dynorphin B) immunoreactive cells and fibres of either peptide have been found in the PAG (Basbaum & Fields 1984). Since immunoreactive β-endorphin has also been localized in nerve terminals within the PAG (Bloom *et al.* 1978) all three opioid peptide families are present in this important 'pain-related' structure and might have specific receptors therein.

There appears to exist considerable evidence that the μ opioid receptor can mediate the antinociceptive actions of opioid alkaloids and opioid peptides at the supraspinal level (Wood 1982). Thus, various μ-receptor specific drugs (morphine, fentanyl, etc.) consistently produce analgesia, upon injection into laboratory animals. In addition, the opioid peptide β-endorphin which has affinity for μ-receptors has been shown to induce a long-lasting analgesia after intracerebroventricular (i.c.v.) injection into rats or mice (for review see Teschemacher 1978). Recently, we found that the larger pro-enkephalin A peptides (peptide E, BAM-22P) also elicited a substantial analgesia in the mouse (Höllt *et al.* 1982). These peptides exhibited a high affinity and selectivity for μ-receptor binding *in vitro* (Garzon *et al.* 1983; Höllt *et al.* 1983). Moreover, they are much more potent in inhibiting the guinea-pig ileum than the mouse vas deferens preparation (see table 1) indicating that they are agonists on μ- rather than on δ-receptors (Lord *et al.* 1977).

As compared to μ-receptors, a putative involvement of δ-receptors in supraspinal analgesia is less-well documented. The endogenous ligands for the δ-receptor, [Met] and [Leu]enkephalins, elicited weak and transient analgesic effects in tail-flick tests upon i.c.v. injection of relatively high amounts (about 200 μg). In many cases, no analgesia was observed (for review see Teschemacher 1978). However, the weak potency of the enkephalins appears to be the result of their rapid degradation; in support of this it has been found that inhibitors of enkephalin degrading enzymes ('enkephalinases') such as thiorphan (Roques *et al.* 1980) can potentiate the analgesic effects of administered enkephalins. Similarly, the presence of enzyme inhibitors in the bioassay preparations (guinea-pig ileum, vas deferens of the mouse, rat and rabbit) increases the inhibitory potency of the enkephalins up to 50-fold (see table 1; McKnight *et al.* 1983). Several δ-receptor specific synthetic enkephalins which are resistant to degradation are potent analgesics. However, the analgesia induced by such a highly specific δ-receptor enkephalin derivative as [D-Ser^{2}-Leu^{5}]enkephalyl-Thr might be due to its much lower affinites to μ- (or ε- ?) receptors, since the highly selective δ-antagonist ICI 154, 129 is not able to reverse

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the antinociceptive effect (Chaillet *et al.* 1983). On the other hand, some indication for a role of δ-receptors in antinociception was found in cross-tolerance studies. Rats chronically infused with the preferential δ ligand [D-Ala^2-D-Leu^5]enkephalin [DADLE] showed a 15-fold tolerance to this peptide in analgesic tests, but only a twofold tolerance to the μ-agonist, sufentanyl (Schulz *et al.* 1981).

There is even less evidence indicating that κ-receptors might be involved in the antinociceptive action of opioids at a cerebral level (i) opioid alkaloids with κ-receptor selectivity such as ethylketocyclazocine (EKC) or bremazocine are inactive when intracerebrally injected into rats or mice (Wood *et al.* 1981; Hayes *et al.* 1983). (ii) Opioid peptides with high selectivity for κ-receptors such as the pro-enkephalin B-derived dynorphins are essentially inactive in inducing analgesia after i.c. injection (Wüster *et al.* 1980; Friedman *et al.* 1981; Herman *et al.* 1980; Höllt *et al.* 1982; see also table 1). The failure to demonstrate analgesic effects of dynorphin$_{1-13}$ has been explained by the short *in vivo* half-life of the administered peptide (Herman *et al.* 1980). This peptide (and also the naturally occurring dynorphin A), however, appear to be resistant to enzymic degradation in isolated tissue preparations, since its potency is not increased in the presence of enzyme inhibitors (Corbett *et al.* 1982; see table 1). Even assuming that dynorphin A might be less resistant to degrading enzymes in brain, it is difficult to explain why peptide E and BAM-P22-peptides which possess a striking homology with the aminoacid sequence and even with the nucleotide acid sequence of the corresponding gene regions (Comb *et al.* 1983) exhibit analgesic effects of relatively long duration (maximum effect at about 20 min, Höllt *et al.* 1982). This would imply that completely different ('non-specific') degrading enzymes would exist for peptide E and dynorphin A: an unlikely hypothesis. Thus, the weight of evidence favours the assumption that dynorphin A (and possibly other pro-enkephalin B (= pro-dynorphin)-derived peptides, see table 1) interact with opioid receptors different from those which might mediate analgesia in the brain. Since all these peptides exhibit a high selectivity for κ-receptors, it is unlikely that κ-receptors are involved in eliciting analgesia at supraspinal centres.

It has been hypothesized that κ-selective opioid alkaloids differ from μ-receptor agonists as regards their characteristic antinociceptive profile: they are active in analgesic tests employing mechanical (paw pressure, tail pinch) or visceral noxious stimuli writhing but not in tests employing cutaneous heat stimuli (hot plate) (Tyers 1980). An argument, however, against this interesting concept is the observation that noxious visceral or mechanical stimuli are, in general, of low intensity. Thus, non-opioid drugs such as aspirin and even pure opioid antagonists like naloxone have been found to be analgesic in the writhing tests. Therefore, the suppression of stronger stimuli such as noxious heat stimuli might be selective for opioid analgesics. In fact, dynorphin A has recently been shown to induce analgesia after i.c. injection into rats when measured in the paw pressure and writhing test, but not in the tail-flick test (Hayes *et al.* 1983; Kaneko *et al.* 1983). However, the dynorphin A-induced analgesia was not susceptible to antagonism by naloxone (Hayes *et al.* 1983). It would be, however, essential to demonstrate an antagonism by naloxone, since strong non-opiate effects of dynorphin A have been reported (Walker *et al.* 1982*a*; Przewłocki *et al.* 1983*a*).

The putative involvement of the ε-receptor in opioid-induced analgesia has not as yet been studied. The prototype agonist β-endorphin induces a long-lasting analgesia after i.c.v. injection which might be mediated, not only via μ-, but also via ε-receptors. Although the ε-receptor was originally postulated to exist on the selective potency of β-endorphin on the rat

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vas deferens (Wüster *et al.* 1979) all pro-enkephalin A-derived opioid peptides possess activity in this preparation. We will show below that the relative potencies of the peptides on the rat vas deferens correlate excellently with their analgesic potencies in the mouse (see figure 1).

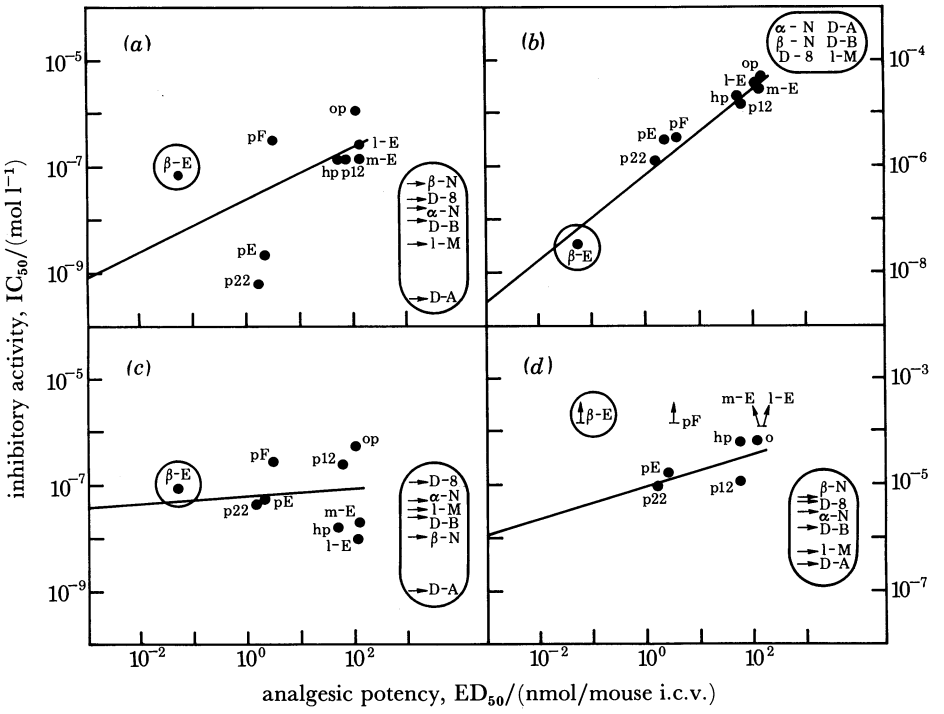

FIGURE 1. Correlation between antinociceptive potency in the mouse and the inhibitory potency on peripheral bioassays of endogenous opioid peptides: (*a*) guinea-pig ileum ($r = 0.26$); (*b*) rat vas deferens ($r = 0.96$; $p < 0.001$); (*c*) mouse vas deferens ($r = 0.06$); (*d*) rabbit vas deferens ($r = 0.44$). Concentrations of opioid peptides that inhibit the muscle twitch by 50% (IC_{50}) and antinociceptive effects in the mouse as measured by the heat radiant tail-flick test are from table 1. Abbreviations: β-E = human β-endorphin; pF = peptide F; pE = peptide E; p22 = BAM-22P; p12 = BAM-12P; hp = heptapeptide = [Met]enkephalyl-Arg^6-Phe^7; op = octapeptide = [Met]enkephalyl-Arg^6-Gly^7-Leu^8; m-E = [Met]enkephalin; l-E = [Leu]enkephalin, α-N = α-neo-endorphin, β-N = β-neo-endorphin; D-8 = $dynorphin_{1-8}$; D-A = dynorphin A; D-B = dynorphin B; l-M = [Leu]morphin. The values of the peptides of the pro-enkephalin B (= pro-dynorphin) family and that of the POMC-peptide β-endorphin are circled. Arrows indicate that these peptides are inactive in a given test system (see also table 1).

(*b*) *Spinal opioid receptors*

In contrast to the brain, only two opioid peptide systems (pro-enkephalin A and pro-enkephalin B (= pro-dynorphin)) have been localized in the mature spinal cord. Cells or fibres containing POMC-derived peptides have not been reported. In microdissected human spinal cord, immunoreactive pro-enkephalin A ([Met]/enkephalin) and pro-enkephalin B (dynorphin A, alpha-neo-endorphin) peptides have been found in highest concentrations in the substantia gelatinosa (Przewłocki *et al.* 1983*b*). A similar distribution of pro-enkephalin A and pro-enkephalin B-producing cells, and fibres within the marginal layer and the substantial gelatinosa in the spinal cord of rats, has been recently reported with even some neurons showing co-existence of pro-enkephalin A and pro-enkephalin B peptides (Basbaum & Fields 1984). In addition, peptides derived from each precursor could be released into superfusates by electrical nerve stimulation (Nyberg *et al.* 1983).

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There are several reports in the literature showing the existence of multiple opioid receptor sites (Fields *et al.* 1980; Członkowski *et al.* 1983). As determined by binding studies *in vitro*, κ-receptor sites were the dominant (50%) type in the human spinal cord followed by μ and rather small amounts of δ sites. In the rat, an even higher percentage of κ sites was found (Gouarderes *et al.* 1982; Członkowski *et al.* 1983).

Consistent with these biochemical demonstrations of multiple opioid binding sites, is a significant literature suggesting a differentiation of receptors on the basis of differential effects of spinally administered opioid alkaloids and peptides.

As in the brain, there is little doubt that μ-receptor agonists can induce analgesia at the level of the spinal cord. This has already been emphasized in the pioneering study of Martin *et al.* (1976) on the spinal dog. μ-Receptor agonists are analgesics in all common test-systems indicating that they can inhibit responses evoked by heat, pressure and visceral stimuli (Tyers 1980). In animals rendered chronically tolerant to morphine, the intrathecally (i.t.) administered opioid peptide β-endorphin displays clear cross-tolerance in analgesic tests indicating that this peptide might interact with μ receptors (Yaksh 1983).

There are also good indications that δ receptors may mediate antinociception in the spinal cord: thus, animals tolerant to morphine are not (or much less) tolerant to the antinociceptive action of the i.t. administered δ agonist DADLE ([D-Ala^2, D-Leu^5]enkephalin) (Yaksh 1983). There is a recent report indicating that i.t. applied DADLE is analgesically active in suppressing cutaneous heat stimuli, but not in inhibiting chemical visceral stimuli (i.e. writhing) (Schmauss & Yaksh 1974). Although this interesting observation indicates a differential site of spinal DADLE action contrasting results have been reported by others (Przewłocki *et al.* 1983*b*).

There is also accumulating evidence that κ-receptors might be involved in spinal antinociception. Wood *et al.* (1981) have shown that in distinction to morphine, there is no loss of the effect of ethylketacyclazocine (EKC) in the spinally transected animal suggesting that the κ-receptor agonists act essentially at the level of the spinal cord. In support of this finding, opioid peptides derived from pro-enkephalin B (dynorphin A, dynorphin$_{1-8}$ and dynorphin B) which display selectivity for κ-receptors induce analgesia when injected into the spinal cord but not into the brain of rodents (Piercey *et al.* 1982; Han & Xie 1982; Przewłocki *et al.* 1983*a*, *c*; Han & Xie 1984; Han *et al.* 1984). The demonstration of the analgesic activity of dynorphin A in the spinal cord of rats was associated with technical problems: thus, dynorphin A was active in rats bearing chronically implanted catheters for 1 to 2 but not for eight days (Han & Xie 1984, Przewłocki *et al.* 1983*a*). Such difficulties might account for the inability of Tung & Yaksh (1982) to obtain analgesia after injection of dynorphin$_{1-13}$ in rats.

Nothing is known concerning the involvement of ε-receptors in spinally induced antinociception. The analgesic characteristics of i.t. administered β-endorphin are very similar to that of morphine (pA_2, cross-tolerance) indicating that its effect might be predominantly exerted via μ-receptors (Yaksh 1983).

(*c*) *Putative interactions between various opioid ligands*

There are several reports indicating that the analgesic effects induced by an opioid peptide or alkaloid might be modified by co-administration of another opioid peptide. Thus, [Leu]-enkephalin has been reported to markedly potentiate the analgesic effects of morphine or [Met]enkephalin, an effect seen at spinal and supraspinal structures (Vaught & Takemori 1978; Larson *et al.* 1980). The dynorphin A fragment dynorphin$_{1-13}$ has the opposite effect:

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it significantly inhibits morphine- or β-endorphin-induced analgesia, despite not having any appreciable analgesic activity itself (Tulunay *et al.* 1981).

This inhibitory effect on analgesia does not, however, necessarily require an opioid-like molecule, since the non-opioid analogue [des-Tyr^1]dynorphin is also able to inhibit morphine-induced analgesia (Walker *et al.* 1982*b*). On the other hand, there is also biochemical evidence for an interaction between different opioid ligands. Thus, *in vitro* binding experiments indicated that dynorphin A inhibits the receptor binding of prototypical μ and δ agonists in a non-competitive manner (Garzon *et al.* 1982). Moreover, there is evidence for an allosteric interaction between μ- and δ-receptor (Rothman & Westfall 1981). In addition, C-terminal fragments of β-endorphin can antagonize β-endorphin-induced analgesia in mice (Lee *et al.* 1980). Of particular interest appears to be that the naturally occurring β-endorphin fragment β-endorphin$_{1-27}$ competitively inhibits the analgesic effect of β-endorphin (Hammonds *et al.* 1984) when coinjected i.c.v. into mice. Although such interaction between opioids might be of great biological significance, the paucity of precise biochemical analyses of the nature of these interactions *in vitro*, does not permit a final judgement on this issue.

4. Correlation between antinociceptive potency in the mouse and activity on peripheral bioassays of the three opioid peptide families

We have employed a pharmacological approach in characterizing the opioid peptides and receptors involved in mediating antinociception, by comparing the potencies of a wide variety of endogenous opioid peptides in inducing analgesia after i.c. injection in the mouse to their potencies in inhibiting electrically induced contractions in four opioid sensitive nerve-muscle preparations *in vitro* (table 1, figure 1). The peptides tested are derived from the three opioid peptide families (POMC, pro-enkephalin A, pro-enkephalin B (= pro-dynorphin)) and appear to be major processing products of their precursors. Their existence in the brain of animals has been demonstrated.

The data for the potencies in the peripheral bioassays are qualitatively and quantitatively similar to those reported by others although not all data have been published elsewhere (Corbett *et al.* 1982; James *et al.* 1984; Quirion & Weiss 1983; Suda *et al.* 1983; Oka & Negishi 1982; McKnight *et al.* 1983). The potencies on the rabbit vas deferens are lower than those reported by others (Corbett *et al.* 1982; Quirion & Weiss 1983) but similar to those reported by Oka & Negishi (1982).

All bioassay experiments have been performed in the absence and in the presence of enzyme-inhibitors and the increased potency has been documented (as potency shift) in table 1. It is quite clear that in the presence of enzyme-inhibitors the smaller peptides of each opioid peptide family (e.g. [Met]enkephalin, [Leu]enkephalin, [Met]enkephalyl-Arg^6-Phe^7, [Met]-enkephalyl-Arg^6-Gly^7-Leu^8, dynorphin$_{1-8}$, α-neo-endorphin, etc.) are markedly increased in potency with greatest effects on the rat vas deferens. In contrast, the longer peptides (β-endorphin, peptide E, peptide F, dynorphin A and [Leu]morphine) did not substantially change their potencies indicating that they are relatively resistant to enzymatic degradation in the tissue preparations. This finding is in line with that reported by McKnight *et al.* (1983) and James *et al.* (1984).

It can be seen from the data in table 1 and figure 1 that the rabbit vas deferens preparation is not responsive to the POMC peptide β-endorphin, whereas the rat vas deferens is not

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sensitive to the pro-enkephalin B (= pro-dynorphin)-derived peptides (with the exception of [Leu]enkephalin), even if enzyme inhibitors are present.

In figure 1, a correlation was made between antinociceptive potencies after i.c. injection in mice with the inhibitory activities in the peripheral bioassays. The IC_{50} values of the peptides in the absence of enzyme inhibitions were chosen for the correlation, since their enzymatic degradation after i.c.v. injection could be anticipated. It is evident from the figure that in the log–log graphs the best correlation was obtained, if antinociceptive potencies were correlated with the potencies of the endogenous peptides on the rat vas deferens. A highly significant correlation coefficient ($r = 0.96$, $p < 0.001$) was obtained with a slope of the straight line of 0.8. In contrast, the correlation of the antinociceptive potencies to the inhibitory activities in the other isolated preparations is not significant.

This correlation is influenced presumably by a comparable degradation of the smaller peptides both in brain and in isolated tissues. Nevertheless, critical for a good correlation appears to be the potency of the enzyme resistant peptides: β-endorphin in relation to the pro-enkephalin A peptides (peptide E, peptide F, BAM-22P). Although not included in the correlation, the lack of the pro-enkephalin B (= pro-dynorphin)-derived peptides is paralleled by the failure of these opioid peptides to inhibit the rat vas deferens. In other bioassays, however, the pro-enkephalin B-peptides display a considerable inhibitory potency.

In view of this correlation, we propose that the antinociceptive action of the endogenous opioid peptides in mouse brain might be mediated via opioid receptors resembling those in the rat vas deferens. These receptors might not be the μ-receptors, since BAM-22P which has a higher potency than β-endorphin on μ-receptors (Höllt *et al.* 1983) (as also indicated by its high potency ratio guinea-pig ileum versus mouse vas deferens; see table 1), is much weaker than β-endorphin in inducing analgesia and in inhibiting the rat vas deferens. Conversely, peptide F which is very weak in the guinea-pig ileum bioassay exhibits substantial antinociceptive potency in mouse brain and inhibitory potency in the rat vas deferens.

The rat vas deferens has been postulated to bear a unique population of opioid receptors (ε-receptors) which selectively bind β-endorphin (Wüster *et al.* 1979). This receptor type, however, also binds the enkephalins (McKnight *et al.* 1983) and essentially all opioid peptides derived from pro-enkephalin A (table 1). The fact, however, that β-endorphin is still by far the most potent endogenous opioid and that the rank order of potencies of the many endogenous opioids on this bioassay is markedly different than on the three others, strongly suggest a distinct population of opioid receptors in the rat vas deferens.

In addition, it might not be unreasonable to assume that opioid receptors via which endogenous opioid peptides elicit analgesia after i.c. injection into mouse brain might be closely related to the ε-receptors in the rat vas deferens. The paucity of biochemical information about the ε-receptor, however, does not presently allow for any further conclusions. It might be of relevance, however, that *in vitro* binding studies in which the putative ε-receptor ligand [^{3}H]β-endorphin has been investigated, significant differences in its binding characteristics and distribution as compared to that of the prototype μ, δ and κ ligands have been found (Law *et al.* 1979; Akil *et al.* 1980; Johnson *et al.* 1982). In addition, previous data have specifically pointed to a function of cerebral β-endorphin in a control of nociceptive thresholds, both tonically and under stress (Millan *et al.* 1980).

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