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Lack of Mixed Agonist-Antagonist Properties of [Gln^8-Gly^{31}]-βh-EP-Gly-Gly-NH_2 and [Arg^{9,19,24,28,29}]-βh-EP in the Rat Vas Deferens Neuroeffector Junction: Studies with Naloxone, β-Funaltrexamine and ICI 174,864

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Abstract—The 1–27 truncated fragment of βh-endorphin (βh-EP) as well as [Gln^8,Gly^{31}]-βh-EP-Gly-Gly-NH_2 or [Arg^{9,19,24,28,29}]-βh-EP exhibited opiate agonist activity in the rat vas deferens bioassay; the potency of these peptides was 3 to 6 times less than that of βh-EP. None of these compounds exhibited any degree of antagonism towards the inhibitory action of βh-EP. Naloxone antagonized and reversed the inhibitory action of βh-EP and its analogues though with varying potencies. The apparent naloxone-pA_2 value for βh-EP was 8·94; that for [Gln^8-Gly^{31}]-βh-EP-Gly-Gly-NH_2 was 8·08 and that for [Arg^{9,19,24,28,29}]-βh-EP was 8·38. β-Funaltrexamine (β-FNA) potently antagonized the inhibitory action of βh-EP following non-equilibrium kinetics. Tissue preincubation with 10 nM β-FNA for 60 min followed by extensive washing caused a 10-fold increase in the βh-EP IC50. However, 10 nM β-FNA caused only a 1·2 increase in the IC50 of [Gln^8,Gly^{31}]-βh-EP-Gly-Gly-NH_2 and a 4·1-fold increase in the IC50 of [Arg^{9,19,24,28,29}]-βh-EP. In contrast, preincubation of the tissue with 3 μM ICI 174,864 did not modify the potency of βh-EP or its structural analogues. However, a 60 min pretreatment with 10 μM β-FNA followed by the addition of 3 μM ICI 174,864 revealed a further decrease in the potency of the opiopeptins compared with tissues exposed to β-FNA alone or ICI 174,864 alone. In conclusion, the inhibitory action of these peptides is remarkably sensitive to β-FNA antagonism; in addition the peptides act as pure opiate agonists in marked contrast with the agonist-antagonist properties described in the CNS.

A keystone in the development of opiate pharmacology was the development of morphine structural analogues with antagonist properties. Naloxone, a prototype of an opiate antagonist, has played a pivotal role in the classification of the opiate receptors both for the morphine-like alkaloids as well as for the opiopeptins (Huidobro-Toro & Way 1985). Due to the fact that naloxone exhibits varying affinities for the opiate receptors and considering that its mode of action to displace the opiopeptins might be different from that of the alkaloids, much effort has been devoted to the synthesis of opioid peptides with antagonist activity. Although an impressive number of enkephalin analogues have been produced in the hope of obtaining competitive enkephalin antagonists,few of them are sufficiently selective and potent (Morley 1980; Cotton et al 1984). Results are most promising in the case of the β-endorphin (βh-EP); a number of substituted or extended analogues exhibit agonist-antagonist properties in CNS pharmacology. Nicolas et al (1984) reported that [Gln^8,Gly^{31}]-βh-EP-Gly-Gly-NH_2 or [Arg^{9,19,24,28,29}]-βh-EP are weak analgesics but at concentrations 10-times lower than those required to cause analgesia, these compounds antagonize the β-endorphin-induced antinociception. In addition, the endogenous 1–27 fragment of β-endorphin antagonized in a dose-dependent fashion the analgesic or endocrine effects of β-endorphin (Hammonds et al 1984; Collado-Escobar et al 1986) or etorphine (Nicholas & Li 1985).

The aim of this investigation was two-fold. We explored as to whether βh-EP$_{1-27}$, [Gln^8,Gly^{31}]-βh-EP-Gly-Gly-NH_2 and [Arg^{9,19,24,28,29}]-βh-EP, compounds with apparent agonist-antagonist properties in the CNS, behave similarly in peripheral autonomic neurons. To test this hypothesis we used the isolated preparation of the rat vas deferens, a tissue of particular interest due to the presence of specific sites for β-endorphin (Lemaire et al 1978; Wüster et al 1979; Miranda et al 1979; Huidobro et al 1980; Huidobro-Toro et al 1982a). In addition, we were also interested in exploring whether μ and δ selective opiate antagonists modify the inhibitory potency of β-endorphin in this tissue.

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Materials and Methods

Tissue preparation; quantification of opioid potency

Opioid properties of βh-EP and structurally related peptides were investigated as inhibitory effects on the twitching response of the prostatic portion of the rat vas deferens. Sprague-Dawley rats (250–300 g) were killed by cervical dislocation. The complete ductus was removed, dissected from surrounding tissues and bissected into two portions. The prostatic half was mounted on a 30 mL bath chamber maintained at 37°C with Krebs Ringer solution buffered with 95% O_2: 5% CO_2 as detailed by Huidobro et al (1980). The ductus were transmurally stimulated with rectangular pulses of 1 ms duration, supramaximal voltage (70 V) and 0·15 Hz. Three or four tissues were tested concurrently. Isometric contractions were recorded on a Grass model 79-D oscillograph. After 1 h of tissue incubation under 0·7 g of basal tension without electrical stimulation, non-cumulative concentration response experiments were performed. The peptides were allowed to remain in contact with the tissues for 5

min, the time required for the opiopeptins to reach a stable blockade of the neurotransmission (see Fig. 1). Following the recording of the inhibitory effect, the electrical stimulation was stopped. The tissues were then rinsed with drug-free buffer solution and allowed to rest for 15 min before electrical stimulation was reassumed to test the action of another concentration of the peptide. A least three concentrations of each opiopeptin were tested to perform a concentration-response study; the median inhibitory concentration (IC50) was obtained by interpopulation using linear regression analysis. Results are expressed as mean ± s.e.; IC50 ratios were determined for each separate preparation dividing the IC50 obtained after drug treatment by the IC50 before drug antagonism.

Agonist-antagonist properties

To examine whether tissue preincubation with 9·2 nM [Gln^8,Gly^{31}]-βh-EP-Gly-Gly-NH_2 of 10 nM [Arg^{9,19,24,28,29}]-βh-EP modified the inhibitory potency of βh-EP, βh-EP concentration-response experiments were performed. Tissues were preincubated with the substituted βh-EP analogues 5 min before addition of varying concentrations of βh-EP; IC50 values were obtained as usual. To further explore whether lower concentrations of the βh-EP analogues exhibited βh-EP antagonism, experiments were performed using a test concentration of 30 nM βh-EP. Tissues were incubated with this concentration of βh-EP either alone or in the presence of 0·1 to 10 nM of the βh-EP analogues.

Studies with antagonists

Naloxone. A fixed concentration of 10 nM was used to antagonize the inhibitory activity of βh-EP and related analogues. Concentration-response curves experiments for each opiopeptin was performed before and following a 5 min incubation of the tissues with 10 nM naloxone. Apparent pA_2 values were derived using the expression

$$pA_2 = -\log[\text{Antagonist}] + \log[\text{IC50 ratio}\ \frac{\text{after}}{\text{before}} - 1].$$

β-Funaltrexamine (β-FNA). Since this antagonist exhibits non-equilibrium μ-selective opioid antagonism, tissues were preincubated with varying concentrations of this compound (0·3,1,3,10,30 or 125 nM) at 37°C for 60 min. Following the incubation, the tissues were rinsed extensively with drug-free Tyrode buffer for the next 20 min as detailed by Huidobro-Toro et al (1982a). A βh-EP concentration-response curve was performed in each preparation before and following pretreatment with β-FNA. As controls, tissues wre incubated with an equivalent volume of saline instead of the drug. In the case of [Gln^8,Gly^{31}]-βh-EP-Gly-Gly-NH_2 and [Arg^{9,19,24,28,29}]-βh-EP concentration-response experiments were performed before and after pretreatment with 10 nM β-FNA.

ICI 174,864. Based on the study of Smith & Carter (1986) 3 μM of this peptide was used to examine for δ-opioid antagonism. Concentration-response experiments for βh-EP, [Gln^8,Gly^{31}]-βh-EP-Gly-Gly-NH_2 and [Arg^{9,19,24,28,29}]-βh-EP were performed before and after tissue preincubation with ICI 174, 864.

In a parallel set of experiments, the action of ICI 174,864 was examined in tissues previously exposed to 10 nM β-FNA for 60 min as detailed above. As controls, tissues were incubated with saline. Concentration-response curves were performed before and after the treatment with β-FNA followed by the addition of ICI 174,864.

Statistics

A two-tailed Student's *t*-test for paired comparisons was used for the assessment of significance. A P value less than 0·05 was accepted as significant.

Source of the peptides

βh-EP and structural analogues were synthesized as described by Zaoral et al (1981) and Yamashiro et al (1982); the peptides were dissolved in distilled water (1 μg μL^{-1}). Ten μg amounts were frozen at −20°C; for each experiment aliquots were freshly thawed. Naloxone, naltrexone and β-funaltrexamine hydrochlorides were provided by Dr W. K. Schmidt, from du Pont & Nemours (Wilmington); 1 mg mL^{-1} solutions were prepared in distilled water. ICI 174,864 was a kind donation from M. Rance; it was dissolved in distilled water as 1 mg mL^{-1} stock solution.

Results

Potency of β-endorphin and related peptides

Human β-endorphin (βh-EP) inhibited the electrically-induced muscle twitching of the vas deferens with a median inhibitory concentration of 50·1 ± 5·1 nM (n = 22). Its natural truncated fragment, βh-EP$_{1-27}$, was 6 fold less potent (Fig. 1). [Gln^8,Gly^{31}]-βh-EP-Gly-Gly-NH_2 was 6-fold weaker as an agonist compared with βh-EP whereas [Arg^{9,19,24,28,29}]-βh-EP was 3-fold less active. The concentration-response curves of these peptides exhibited parallelism. In as much as there is animal variability, IC50 values were determined for each set of experiments separately. Tables 1 and 2 summarize the results.

Lack of βh-endorphin antagonism by the βh-EP derivatives

Treatment with 9·2 nM [Gln^8,Gly^{31}]-βh-EP-Gly-Gly-NH_2 or 10 nM [Arg^{9,19,24,28,29}]-βh-EP did not significantly modify the

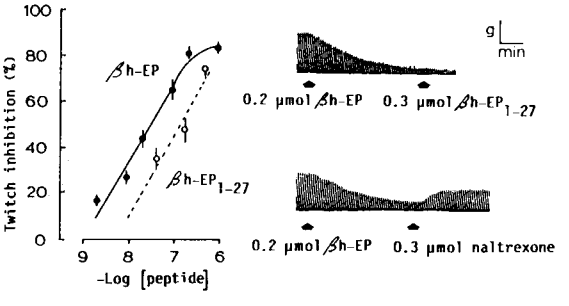

FIG. 1. Opioid activity of βh-EP-(1–27) in the rat vas deferens neurotransmission. Left panel. βh-EP and βh-EP-(1–27) concentration-response curves. Eight different preparations were used to assay the potency of βh-EP, four for βh-EP-(1–27). Symbols indicate the mean percentage of twitch inhibition caused by the application of peptides, bars indicate the s.e.m. The IC50 for βh-EP was 50·1 ± 5·1 nM (n = 22); that for βh-EP-(1–27) was 290 ± 65 nM (n = 4). Right panel. Lack of βh-EP antagonism by βh-EP-(1–27). At the arrows application of 0·2 μM βh-EP, followed by either 0·3 μM βh-EP-(1–27) (upper tracing) or 0·3 μM naltrexone (lower tracing). Both recordings were obtained from the same preparation.

Table 1. Potency of βh-EP in the rat vas deferens in the presence of [Gln^8,Gly^{31}]-βh-EP-Gly-Gly-NH_2 and [Arg^{9,19,24,28,29}]-βh-EP.

	βh-EP IC50 (nM) Mean ± s.e.	
Pretreatment		
Saline	50·1 ± 5·1	(22)
+ 9·2 nM [Gln^8,Gly^{31}]-βh-EP-Gly-Gly-NH_2	56·8 ± 5	(5)
+ 10 nM [Arg^{9,19,24,28,29}]-βh-EP	47·5 ± 9	(8)

βh-EP IC50 (Table 1). Larger concentrations of the peptides could not be examined since they exhibited some degree of βh-EP-like agonist activity. Likewise, lower concentrations of each of these peptides did not significantly modify the inhibitory activity induced by a challenge concentration of 30 nM βh-EP. In a series of six separate experiments, 30 nM βh-EP produced 63 ± 5% inhibition of the twitching response. In the presence of 0·1, 0·3, 1,3 or 10 nM [Arg^{9,19,24,28,29}]-βh-EP, challenge with 30 nM βh-EP inhibited twitch by 66 ± 7, 68 ± 4, 62 ± 3, 59 ± 4 and 59 ± 3%, respectively. Similar results were obtained when tissues were challenged with 30 nM βh-EP in the presence of 0·9, 2·7, 9·2 and 27 nM [Gln^8,Gly^{31}]-βh-EP-Gly-Gly-NH_2. In addition, Fig. 1 shows that in contrast to naltrexone, an equimolar concentration of βh-EP_{1-27} did not reverse the inhibitory action of βh-EP.

Effect of antagonists

Naloxone. This narcotic blocker drug reduced inhibitory activity of the several opiopeptins tested although with different potencies. Addition of 10 nM naloxone displaced to the right in a parallel fashion the βh-EP concentration response-curve increasing its IC50 by about 10-fold. However, 10 nM naloxone antagonized to a lesser extent the inhibitory potency of [Gln^8,Gly^{31}]-βh-EP-Gly-Gly-NH_2 and [Arg^{9,19,24,28,29}]-βh-EP (Table 2A). The apparent naloxone-βh-EP pA_2 value was 8·94, that of the other peptides was correspondingly less (Table 2A). Furthermore, the inhibitory activity of βh-EP was rapidly reversed by naltrexone but not by βh-EP_{1-27} (Fig. 1).

β-Funaltrexamine (β-FNA). This non-competitive selective μ-opioid antagonist, displaced to the right the concentration-response curves of βh-EP. Following an hour of tissue incubation with 3 nM β-FNA, the βh-EP concentration-response curve was shifted about 3-fold in a parallel fashion to the right. However, increasing the concentration of β-FNA to 10 nM, caused a 10-fold non-parallel shift of the βh-EP concentration-response curve (Fig. 2). A further increase of β-FNA markedly reduced the slope of the concentration-response curve.

β-FNA also blocked the inhibitory activity of [Arg^{9,19,24,28,29}]-βh-EP. Consistent with the naloxone results, this drug did not modify the potency of the substituted βh-EP peptides to the same extent. Whereas the potency of [Gln^8,Gly^{31}]-βh-EP-Gly-Gly-NH_2 was unaffected by pretreatment with 10 nM β-FNA, that concentration of the antagonist caused a 4-fold increase in the IC50 of [Arg^{9,19,24,28,29}]-βh-EP (Table 2B).

ICI 174,864. Tissue preincubation with 3 μM of the pentapeptide did not change the potency of the three opiopeptins examined (Table 2C).

Antagonism of ICI 174,864 in tissues pretreated with 10 nM β-FNA

To examine whether alkylation of μ-opiate receptors unmasked βh-EP activity mediated via the δ-opiate receptor, tissues were incubated with 10 nM β-FNA for 1 h followed by the construction of a βh-EP concentration-response curve in the presence of 3 μM ICI 174,864. The potency of βh-EP that decreased about 10-fold by βh-EP alone and was unaffected by ICI 174,864 alone, decreased 24.8-fold when ICI 174,864 was tested in tissues pretreated with β-FNA. Likewise, the potency of [Gln^8,Gly^{31}]-βh-EP-Gly-Gly-NH_2 and [Arg^{9,19,24,28,29}]-βh-EP was further decreased by pretreatment with β-FNA in the presence of 3 μM ICI 174,864. Results of these experiments are summarized in Table 3.

Table 2. Comparative potency of naloxone, β-Funaltrexamine (β-FNA) and ICI 174,874 to antagonize the inhibitory activity of βh-EP and related peptides in the rat vas deferens. (Mean IC50 (nM) ± s.e.)

	Control	After saline	Ratio (a)	pA_2
βh-EP (control (4)	33·5 ± 8·1	24·6 ± 8·5	0·66 ± 0·13	
Exp. A		After 10 nM naloxone		
βh-EP	50·1 ± 5·1 (22)	498·3 ± 56 (6)	9·9	8·94
[Gln^8,Gly^{31}]-βh-EP-Gly-Gly-NH_2 (6)	318·0 ± 41	699·0 ± 108	2·2*	8·08
[Arg^{9,19,24,28,29}]-βh-EP (6)	141·3 ± 19	485·9 ± 80	3·4*	8·38*
Exp. B		After 10 nM β-FNA		
βh-EP (11)	56·5 ± 6·2	487 ± 72	9·9 ± 1·6	
[Gln^8,Gly^{31}]-βh-EP-Gly-Gly-NH_2 (6)	806·5 ± 148	898 ± 149	1·2 ± 0·21**	
[Arg^{9,19,24,28,29}]-βh-EP (7)	275·5 ± 46	1240 ± 269	4·1 ± 0·71**	
Exp. C		After 3 μM ICI 174,864		
βh-EP (3)	54·9 ± 17	69·3 ± 41	1·0 ± 0·4	
[Gln^8,Gly^{31}]-βh-EP-Gly-Gly-NH_2 (4)	812 ± 98	1031 ± 386	1·2 ± 0·3	
[Arg^{9,19,24,28,29}]-βh-EP (4)	183 ± 50	141 ± 29	0·95 ± 0·35	

Numbers in parentheses indicates the times the experiment was repeated in different tissues.
(a) IC50 ratios are mean values of the IC50 ratios obtained for each particular tissue by dividing IC50 after treatment by IC50 before treatment.
* $P < 0·05$, ** $P < 0·01$ compared with IC50 ratio obtained for βh-EP under the same conditions.

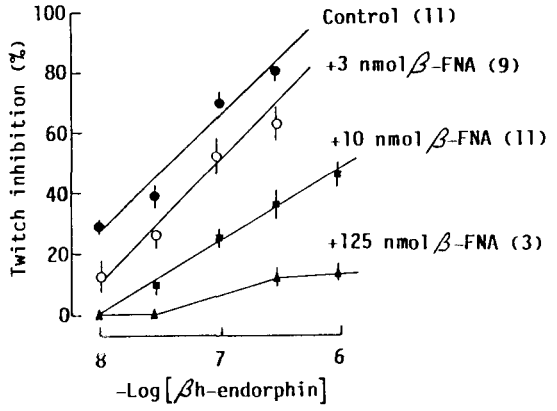

FIG. 2. Antagonism of the inhibitory activity of β-endorphin by β-funaltrexamine (β-FNA). Concentration-response curves to β-endorphin were constructed before and after tissue exposure to varying concentration of β-FNA. Tissues were incubated with β-FNA for 60 min at 37°C followed by 20 min extensive rinsing before a second β-endorphin concentration-response experiment. The βh-EP IC50 before and after incubation with 0·3 μM β-FNA was 41·8 ± 21·6 versus 18·2 ± 7·5 nM (n = 3); that following 3 nM β-FNA was 50·8 ± 10·7 versus 146·2 ± 26 nM (n = 10) and that following exposure to 10 nM β-FNA was 56·5 ± 6·2 versus 487 ± 72 nM (n = 11). In control tissues exposed to saline instead of β-FNA the βh-EP IC50 was 33·5 ± 8·1 versus 24·6 ± 8·5 nM (n = 4).

Discussion

Marked differences were recognized when the analgesic potency of [Gln^8,Gly^{31}]-βh-EP-Gly-Gly-NH$_2$, [Arg^{9,19,24,28,29}]-βh-EP and βh-EP$_{1\text{-}27}$ was compared with the binding affinity of those compounds to brain membranes or to the present results in the vas deferens bioassay. In the first place, βh-EP is about 6-fold more potent as an analgesic than [Gln^8,Gly^{31}]-βh-EP-Gly-Gly-NH$_2$, and 50-fold more potent than βh-EP$_{1\text{-}27}$ (Nicolas et al 1984). In the vas deferens, both [Gln^8,Gly^{31}]-βh-EP-Gly-Gly-NH$_2$ and βh-EP$_{1\text{-}27}$ are about 6-fold less potent than βh-EP. In second place, while all three opioid peptides show agonist-antagonist properties in the CNS, none exhibited antagonist activity in the vas deferens. In this regard, [Gln^8,Gly^{31}]-βh-EP-Gly-Gly-NH$_2$ was reported to be about 40-fold more potent than βh-EP$_{1\text{-}27}$ as a βh-EP antagonist and at least 200 times more potent than naloxone in inhibiting the β-EP-induced analgesia (Hammonds et al 1984).

Opiate receptor subtypes are a matter of controversy in the rat vas deferens. Based on the finding that morphine and related alkaloids (prototype μ-opiate agonists) and the enkephalins (putative δ-opiate agonists) are inactive in this bioassay (Lemaire et al 1978; Miranda et al 1979), Wüster et al (1979) postulated that the potent opiate activity of β-endorphin is due to the activation of a novel receptor subtype termed the E-opiate receptor. Alternative interpretations argue that the lack of δ-opiate activity of the enkephalins is due to the existence of abundant proteolytic enzymatic activity in this tissue (C. H. Li personal communication). The lack of opiate agonist activity of morphine could be explained invoking partial agonism and a low μ-opiate receptor reserve (Smith & Rance 1983; Smith & Carter 1986).

It was of considerable interest therefore to examine in greater detail the pharmacology of β-endorphin following selective opiate receptor blockade. In addition, we characterized some pharmacological properties of [Gln^8,Gly^{31}]-βh-EP-Gly-Gly-NH$_2$ and [Arg^{9,19,24,28,29}]-βh-EP following opiate receptor blockade by naloxone, β-FNA and ICI 174,864 (Cotton et al 1984). The βh-EP-induced inhibitory activity in the rat vas deferens was blocked with high affinity by both naloxone and β-FNA, drugs with great specificity for the opiate receptors but markedly different selectivity and pharmacodynamical properties. Whereas naloxone is a competitive universal opiate antagonist, β-FNA acts as a non-equilibrium μ-selective opiate blocker (Takemori et al 1981; Huidobro-Toro et al 1982b). At a fixed concentration of 10 nM, both antagonists proved equipotent in antagonizing the inhibitory activity βh-EP, and [Arg^{9,19,24,28,29}]-βh-EP. Low concentrations of β-FNA produced a parallel rightward displacement of the βh-EP concentration-response curve; increasing the concentration of β-FNA caused a progressively non-parallel shift, reminiscent of the non-equilibrium antagonism of phenoxybenzamine at the α-adrenoceptor. Interestingly, β-FNA is markedly more potent in the rat ductus than in the guinea-pig ileum as an opiate antagonist (Huidobro-Toro et al 1982b). Furthermore, ICI 174,864 which proved inactive itself as a βh-EP antagonist, revealed some degree of blocking activity following tissue exposure to 10 nM β-FNA. We interpret this finding as indicating that following alkylation of a substantial fraction of μ-like opiate receptors by β-FNA, a small δ-opiate receptor population is unmasked. [Gln^8,Gly^{31}]-βh-EP-Gly-Gly-NH$_2$ is the most sensitive of the peptides to the blocking action of ICI 174,864 thus indicating that this compound, which is the least sensitive to naloxone and β-FNA blockade, is the most extensively blocked by ICI 174,864. A plausible interpretation of this finding is that [Gln^8,Gly^{31}]-βh-EP-Gly-Gly-NH$_2$ has the most δ-opiate receptor efficacy.

The present results are thus compatible with the notion that the rat ductus contains a predominant μ-opiate receptor population sensitive to naloxone and β-FNA and a much smaller δ-opiate receptor population. The lack of βh-EP antagonism by ICI 174, 864 may be explained by the presence

Table 3. Effect of 3 μM ICI 174,864 on the potency of βh-EP and related peptides following a 60 min pre-incubation with 10 nM β-Funaltrexamine (β-FNA). (Mean IC50 (nM) ± s.e.)

	Control	after β-FNA + ICI 174,864	Ratio
βh-EP (8)	26·0 ± 3·6	630 ± 127	24·8 ± 4·8
[Gln^8,Gly^{31}]-βh-EP-Gly-Gly-NH$_2$ (4)	812 ± 98	3306 ± 175	4·6 ± 1·0*
[Arg^{9,19,24,28,29}]-βh-EP (4)	497 ± 82	3534 ± 359	8·1 ± 2·1*

* $P < 0.01$ compared with IC50 ratio obtained for βh-EP under the same conditions.

of a small δ-receptor population in the rat ductus compared with the predominant μ-population which masks the δ-opiate receptor mediated effects. Only after a significant fraction of the μ-opiate receptor population is alkylated, can the δ-receptor-mediated effects be appreciated. The same reasoning explains why there is no agonist-antagonist effects in this tissue despite the existence of a receptor population. Alternatively, we admit that the E-opiate receptor is remarkably similar to the μ-opiate receptor in terms of the pharmacology of the opiate antagonist drugs, and that this site has great affinity for β-FNA.

Differences between the central and peripheral pharmacology could be rationalized postulating that the agonist-antagonist effects in the CNS are mediated by a non μ-like receptor, probably a δ-receptor site at which [$Arg^{9,19,24,28,29}$]-βh-EP and specially [Gln^{8},Gly^{31}]-βh-EP-Gly-Gly-NH_2 have a higher affinity than βh-EP.

Little is as yet known about the physiological significance of βh-EP in the vas deferens. Recent studies demonstrated the presence of immunoreactive pro-opiomelanocortin peptides and of pro-opiomelanocortin mRNA in the adult rat testis (Margioris et al 1983; Pintar et al 1984). Furthermore, the βh-EP present in the testis corresponds to the non-acetylated form (Margioris et al 1983). Thus, it is possible that the βh-EP present in the testis plays a role in the transport of sperms modulating the release of neurotransmitters from adrenergic nerve varicosities and thereby regulate the muscular tone of the ductus.

The molecular basis underlying the competitive interaction between naloxone and βh-EP or β-FNA and βh-EP is as yet not clear, even though this is a classical and reproducible observation (see review by Huidobro-Toro & Way 1985). In this connection, it is surprising that the *N*-acetyl derivative of βh-EP is fully inactive as an opiate agonist or as an antagonist in the vas deferens (Huidobro-Toro et al 1982a). Based on these results, it was no surprise to us that βh-EP_{1-27}, [Gln^{8},Gly^{31}]-βh-EP-Gly-Gly-NH_2 or [$Arg^{9,19,24,28,29}$]-βh-EP did not antagonized the inhibitory action of βh-EP on neurotransmission in the rat vas deferens.

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