Evidence for lack of modulation of μ -opioid agonist action by δ -opioid agonists in the mouse vas deferens and guinea-pig ileum

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1 There is evidence from *in vivo* studies for an interaction of μ - and δ -opioid ligands. In the present work this concept has been investigated using the mouse vas deferens and guinea-pig ileum myenteric plexus-longitudinal preparations.

2 In field stimulated vasa deferentia of the mouse, co-administration of sub-effective concentrations of the δ -opioid agonist [D-Pen²,D-Pen⁵]enkephalin (DPDPE) and [Met⁵]- or [Leu⁵]enkephalin had no effect on the dose-response curves of the μ -agonists [D-Ala²,MePhe⁴,Gly-ol⁵]enkephalin (DAMGO) and morphine. Similarly, the δ -opioid agonists did not alter the potency of morphine and DAMGO when added at different times prior to the μ -opioid agonists, or when EC₅₀ concentrations of δ -opioid ligands were co-administered. Compounds with preferred activity for the putative δ_1 -(DPDPE) or δ_2 - ([D-Ala²,Glu⁴]deltorphin II (Delt II)) opioid receptors were ineffective in this respect.

3 The guinea-pig ileum contains δ -opioid receptors. No function of these receptors in mediating blockage of field-stimulated contractions was observed with ligands having affinity for the putative δ_1 or δ_2 subtypes nor were the agonists able to modulate responses to μ -opioid ligands in this tissue.

4 The results demonstrate that modulation of μ -opioid agonists by δ -opioid agonists does not occur in the isolated peripheral tissues examined. Thus the findings do not support the concept of a functional coupling of opioid receptors, though the results may be explained by differences between opioid systems in the brain and peripheral tissues examined.

Keywords: μ -Opioid receptor; δ -opioid receptor; opioid receptor subtypes; μ/δ interactions; β -endorphin; mouse vas deferens; guinea-pig ileum

Introduction

The ability of δ -opioid agonists to modulate the *in vivo* actions of μ -opioid agonists to promote or decrease their activity has been demonstrated (for review see Traynor & Elliott, 1993). The interactions have been reported for antinociceptive⁴ tests (Vaught & Takemori, 1979; Lee *et al.*, 1980; Jiang *et al.*, 1990a) but also extend to other activities such as bladder contraction (Sheldon *et al.*, 1989), gut propulsion (Heyman, 1987), EEG and EEG spectral power (Stamidis & Young, 1992) and antitussive activity (Kamei *et al.*, 1993). These effects are observed following the administration of a sub-effective dose of certain δ -opioid agonists. Thus the δ -opioid [D-Pen²,D-Pen⁵]enkephalin (DPDPE) affords an increase in the potency of μ -opioid ligands whilst the δ -opioid agonist [Met⁵]enkephalin shifts dose-response curves for the μ -opioid agonists to the right. Both the positive and negative modulatory actions of δ -opioid agonists are prevented by pretreatment with the selective δ -opioid antagonist, ICI 174,864 (N,N-diallyl-Tyr-Aib-Phe-Leu-OH) (Jiang *et al.*, 1990a).

Interestingly not all μ -opioid ligands are responsive to modulation. Thus using the mouse tail-flick assay DPDPE, administered either i.c.v. or s.c., increases the potency of morphine, normorphine, levorphanol, methadone and codeine but not the generally more efficacious μ -opioid agonists [D-Ala²,MePhe⁴,Gly-ol⁵]enkephalin (DAMGO), [NMePhe³,D-Pro⁴]morphiceptin (PLO17), phenazocine, sufentanil or etorphine (Vaught & Takemori 1979; Heyman *et al.*, 1989). On the other hand, potentiation of the activity of the μ -opioid agonists PLO17 and DAMGO by use of higher (ED₅₀) levels of δ -opioid ligands, administered intrathecally, has been reported in the rat hot plate test (Malmberg & Yaksh, 1992). The reasons for this apparent discrepancy

between observations is unknown but may be related to species differences and/or the route of drug administration employed.

The concept of interacting μ - and δ -opioid systems has received support from mathematical analyses of ligand binding assays (Barrett & Vaught, 1983; Demoliou-Mason & Barnard, 1986; Rothman *et al.*, 1988). Following these studies δ -opioid receptors have been divided into so-called μ -complexed and μ -non-complexed types. Biochemical evidence has been provided by the ability of both μ - and δ -opioid ligands to inhibit the covalent linking of β -endorphin to rat striatal membranes (Schoffelmeer *et al.*, 1990). In addition recent work with newly developed antagonists lends support to the presence of a heterogeneous δ -opioid receptor population, leading to a division into so-called δ_1 and δ_2 subtypes (Jiang *et al.*, 1991). However, the relationship between the two definitions of δ -opioid subtypes is unclear (Traynor & Elliott, 1993).

Interactions between co-administered μ - and δ -opioid agonists could be of major clinical importance. It may be possible to overcome tolerance and the ceiling effects of certain lower efficacy μ -opioid ligands. For example it has been shown that while DPDPE and [Leu⁵]enkephalin increase the potency of morphine, if either compound is co-administered with morphine then it is only the amount of morphine administered that determines the rate of development of tolerance (Jiang *et al.*, 1990b). To investigate the phenomenon further it would be helpful if such interactions could be observed *in vitro*. This study examines whether two tissue preparations which respond to opioid agonists by an inhibition of electrically-induced contractions, namely the mouse vas deferens and the guinea-pig ileum myenteric plexus-longitudinal muscle, might be suitable models to address this issue. The mouse vas deferens contains a predominantly δ -opioid receptor population, which recent studies

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suggest may be the putative δ_2 subtype (Wild *et al.*, 1993), with μ - and κ -opioid receptors also present (Lord *et al.*, 1977). In this tissue evidence for an interaction between μ - and δ -opioid receptors has been obtained by examining the ability of various ligands to protect the δ -site from alkylation by the affinity ligand β -chlornaltrexamine (Sheehan *et al.*, 1986). The guinea-pig ileum myenteric plexus-longitudinal muscle contains functional μ - and κ -opioid receptors (Chavkin *et al.*, 1982). Whilst δ -opioid receptors have been shown in ligand binding (Leslie *et al.*, 1980) and electrophysiological studies (Egan & North, 1981) ligand occupation of these sites does not lead to a blockade of electrically-induced contractions. Therefore we have considered whether this δ -opioid receptor population might be able to modulate the actions of μ -opioid agonists. Some of the findings of this study have appeared in preliminary form (Elliott & Traynor, 1994).

Methods

Bioassays

Male Dunkin-Hartley guinea-pigs (300–450 g) (David Hall, Burton-on-Trent) were killed by cervical dislocation and segments of ileum were removed and placed in Krebs solution containing (mM): NaCl 118, KCl 4.7, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 2.6, KH_2PO_4 1.2, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 1.2, NaHCO_3 25 and glucose 11. Male CSI mice (25–30 g) (Nottingham University Medical School) were killed by decapitation and vasa deferentia removed and placed in Krebs solution minus $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$.

Mouse vasa deferentia and myenteric plexus-longitudinal muscle preparations of the guinea-pig ileum were set up for field stimulation as previously described (Traynor *et al.*, 1987). Concentration-effect curves for the inhibition of electrically-induced contractions were constructed by cumulative addition of agonists to the bathing fluid. Initially concentration-effect curves for the δ -opioid agonists were obtained. Concentration-effect curves for μ -opioid agonists were then constructed in the absence and presence of a predetermined concentration (either sub-effective or EC_{50}) of δ -opioid agonist. Antagonist equilibrium dissociation constants (K_c , nM) were determined from the ratios of EC_{50} values for agonists in the absence or presence of antagonist (added 15 min prior to the redetermination of agonist concentration-response curves) using the formula $K_c = [\text{antagonist}]/\text{dose ratio} - 1$ (Kosterlitz & Watt, 1968). Statistical comparisons were made where appropriate by Student's *t* test, where $P < 0.05$ was considered significant.

Materials

The following drugs and peptides were used: morphine, naloxone, β -endorphin, [Met⁵]- and [Leu⁵]enkephalin, [D-Ala², MePhe⁴, Gly-o⁵]enkephalin (DAMGO) and [D-Pen², D-Pen⁵]enkephalin (DPDPE) (Sigma); [D-Ala², Glu⁴]deltorphin II (Delt II) (Peninsula); cyprodime and naltrindole (Research Biochemicals).

Results

Mouse vas deferens

The δ -opioid receptor selective ligands, DPDPE (Mosberg *et al.*, 1983) and [D-Ala², Glu⁴]deltorphin II (Delt II) (Erspamer *et al.*, 1989) and [Met⁵]- and [Leu⁵]enkephalin were full agonists in the field stimulated mouse vas deferens preparation with potencies (EC_{50} values) of 0.70 ± 0.06 nM, 0.28 ± 0.03 nM, 5.7 ± 1.2 nM and 6.6 ± 0.9 nM respectively (Figure 1). Concentrations of δ -opioid agonists affording $< 10\%$ inhibition were chosen as sub-effective concentrations, i.e., 0.1 nM for DPDPE and Delt II or 1.0 nM for

[Met⁵]- and [Leu⁵]enkephalin for subsequent experiments. Concentration-effect curves were obtained for the μ -agonists DAMGO and morphine and repeated in the presence of a co-administered δ -opioid ligand at the chosen sub-effective concentration. There was no significant difference in the potencies for the μ -opioid ligands under the two conditions (Table 1), nor in the slope of the concentration-effect curves (Figure 2). Similarly no attenuation or enhancement of the EC_{50} for the μ -agonists was observed when the δ -opioid agonists were added 2, 4 or 15 min prior to the redetermination of the μ -opioid agonist concentration-effect curve (Table 1).

DPDPE added at a concentration causing 50% inhibition of the electrically evoked twitch also failed to alter significantly the potency of the μ -opioid agonists, morphine (EC_{50} 180 ± 13 nM in the absence of DPDPE compared to 212 ± 22 nM in the presence of DPDPE) and DAMGO (EC_{50}

Figure 1 Inhibition of electrically-induced contractions of the mouse vas deferens by DPDPE (●), Delt II (□), [Met⁵]enkephalin (○) and [Leu⁵]enkephalin (■). Values are means $\pm$ s.e.mean where $n \geq 3$. For abbreviations in this and subsequent legends, see text.

Table 1 Potency of μ -ligands in the mouse vas deferens in the absence and presence of sub-effective concentrations of δ -agonists added at different times prior to the μ -agonist

μ -Opioid agonist	δ -Opioid agonist	Time (min)	IC_{50} (nM)
DAMGO			15.3 ± 1.7
DAMGO	DPDPE	0 (coadministered)	12.1 ± 1.1
		2	12.1 ± 0.9
		4	11.4 ± 1.6
		15	18.3 ± 1.3
	Delt II	0 (coadministered)	16.0 ± 2.4
		2	10.5 ± 1.4
		4	10.5 ± 0.6
		15	13.2 ± 0.7
Morphine			180 ± 13
Morphine	DPDPE	0 (coadministered)	200 ± 16
		2	130 ± 21
		4	122 ± 17
		15	195 ± 30
	Delt II	0 (coadministered)	150 ± 6.5
		2	192 ± 44
		4	161 ± 24
		15	248 ± 60
	[Leu ⁵]enkephalin	0 (coadministered)	233 ± 26
	[Met ⁵]enkephalin	0 (coadministered)	182 ± 12

Values are means $\pm$ s.e.mean where $n \geq 3$. None of the results was significantly different from additive where $P < 0.05$.

15.3 ± 1.7 nM in the absence of DPDPE compared to 14.2 ± 3.6 nM in the presence of DPDPE) when co-administered. The same lack of effect was observed with Delt II and also [Met⁵]- and [Leu⁵]enkephalin.

β -Endorphin has similar affinity for μ - and δ -opioid receptors (Kosterlitz *et al.*, 1986). This compound was a full agonist in the mouse vas deferens affording an EC_{50} of 31.3 ± 2.7 nM. The dose-effect curve for β -endorphin was shifted to the right in the presence of the non-selective opioid antagonist naloxone (200 nM), having an apparent K_i for naloxone of 26.0 ± 4.3 nM, indicative of an action at δ -opioid receptors (Figure 3). This was confirmed by the effectiveness of the δ -opioid receptor selective antagonist naltrindole (Rogers *et al.*, 1990) in antagonizing the β -endorphin response, affording a K_i of 0.86 ± 0.10 nM, and the lack of antagonism by cyprodime, a selective μ -opioid antagonist (Schmidhammer *et al.*, 1989), affording a $K_i > 300$ nM (Figure 3).

Figure 2 Inhibition of electrically-induced contractions of the mouse vas deferens by DAMGO (closed symbols) and morphine (open symbols) alone (circles) or coadministered with sub-effective concentrations of DPDPE (squares) or Delt II (triangles). Values are means $\pm$ s.e.mean where $n \geq 3$.

Figure 3 Concentration-effect curves for β -endorphin in the electrically stimulated mouse vas deferens in the absence (●) or presence of 200 nM naloxone (■), 300 nM cyprodime (□) or 6 nM naltrindole (○). Values are means $\pm$ s.e.mean where $n \geq 3$.

Guinea-pig myenteric plexus-longitudinal muscle

DPDPE ($EC_{50} = 4740$ nM) and Delt II ($EC_{50} = 2180$ nM) were very weakly active in inhibiting the electrically-evoked twitch of the longitudinal muscle in this preparation compared to DAMGO ($EC_{50} = 8.3$ nM) and morphine ($EC_{50} = 100$ nM) (Figure 4). Co-administration of 0.3μ M DPDPE, a concentration which was just sub-effective, with the μ -opioid ligands caused no alteration in the potency of DAMGO ($IC_{50} = 8.3 \pm 2.0$ nM in the absence of DPDPE and 7.3 ± 1.2 nM in the presence of DPDPE) or morphine ($EC_{50} = 100 \pm 34$ nM in the absence of DPDPE and 92 ± 37 nM in the presence of DPDPE). A sub-effective concentration of Delt II was also unable to modulate the potency of the μ -opioid ligands. Similarly no change was observed when either DPDPE or Delt II was used at a concentration which inhibited 50% of the twitch response (data not shown).

Discussion

The results show that in the mouse vas deferens, modulation of the action of μ -opioid agonists by δ -opioid agonists cannot be demonstrated. This applies to both peptide and non-peptide μ -opioid agonists and with either sub-effective levels of δ -opioid agonists or concentrations of δ -opioid agonists which by themselves cause 50% inhibition of the electrically-induced twitch. This is perhaps surprising since both μ - and δ -opioid receptors exist in this tissue and indeed have been shown to exist on the same neurone (Rogers & Henderson, 1990). On the other hand, the results do support the suggestion of an anatomical but not functional coupling of the receptors (Sheehan *et al.*, 1986). In contrast, in the brain μ - and δ -opioid receptors may be functionally coupled since interactions have been shown to occur in the inhibition of the release of neurotransmitters from brain slice preparations. Thus the selective δ -opioid, DPDPE, blocks the morphine (μ -mediated) inhibitions of D_1 dopamine receptor-stimulated cyclic AMP efflux (Schoffelmeier *et al.*, 1987). The results may suggest that differences exist in opioid receptor systems between the brain and periphery. Indeed there is evidence that the δ -opioid receptor populations in the brain and periphery are different. For example, the conformationally constrained peptide [D-Ala²,(2R,3S)- ∇^5 Phe⁴,Leu⁵]enkephalin (CP-OH) is very weakly active in the mouse vas deferens

Figure 4 Concentration-effect curves for selective μ - and δ -opioid agonists in field stimulated guinea-pig myenteric plexus-longitudinal muscle, DAMGO (●), morphine (□), Delt II (○) and DPDPE (■). Values are means $\pm$ s.e.mean where $n \geq 3$.

although it has high affinity for the δ -opioid receptor in rat brain and can modulate the antinociceptive effects of morphine (Shimohigashi *et al.*, 1987; 1988). Additionally, whereas the tritiated ligand [^3H]-*p*-Cl-DPDPE has similar affinity for δ -opioid receptors in the mouse vas deferens and rat brain, the methyl ester of CP-OH (CP-OMe) has a 33-fold lower affinity for the δ -opioid receptors in the mouse vas deferens than those in brain (Vaughn *et al.*, 1990).

Recent *in vivo* evidence has suggested a division of the δ -opioid receptor population in the central nervous system into putative δ_1 and δ_2 subtypes. DPDPE and [D-Ala²,D-Leu⁵]enkephalin (DADLE) are thought to activate the δ_1 subtype, and are antagonized by 7-benzylidenenaltrexone (BNTX) and [D-Ala²,Leu⁵,Cys⁶]enkephalin (DALCE). Conversely, Delt II and [D-Ser²,Leu⁵,Thr⁶]enkephalin (DSLET) are agonists at the δ_2 subtype while naltriben (the benzofuran analogue of naltrindole) and naltrindole-5'-isothiocyanate (5'-NTII) are antagonists (for example see Sofuoglu *et al.*, 1991; Jiang *et al.*, 1991). Furthermore, a lack of cross-tolerance between DPDPE and Delt II or DSLET is consistent with this classification (Sofuoglu *et al.*, 1991; Mattia *et al.*, 1991). In addition, ligand-binding experiments add credence to the existence of multiple δ -opioid subtypes (Negri *et al.*, 1991; Sofuoglu *et al.*, 1992; Portoghesi *et al.*, 1992). There is evidence to suggest that the δ -opioid receptor involved in modulating centrally μ -opioid mediated effects is of the putative δ_2 subtype (Porreca *et al.*, 1992; Kamei *et al.*, 1993). However, in the mouse vas deferens, experiments with selective δ -opioid subtype antagonists point to a single homogeneous δ -opioid receptor population (Wild *et al.*, 1993). Our finding that no difference in effect is seen with either a putative δ_1 -opioid agonist (i.e., DPDPE) or a putative δ_2 -opioid agonist (i.e., Delt II) would support this.

The endogenous opioid peptide, β -endorphin, has similar affinity for μ - and δ -opioid receptors (Kosterlitz *et al.*, 1986). The peptide has been demonstrated to bind to both μ - and δ -sites simultaneously in rat brain membrane preparations

(Schoffelemeier *et al.*, 1990) and can be blocked by both μ - and δ -opioid antagonists, providing evidence for receptor interaction. However, this does not appear to be the case in the mouse vas deferens since our studies with antagonists indicate that β -endorphin is exerting its actions solely through activation of δ -opioid receptors.

In the myenteric plexus-longitudinal muscle preparation of the guinea-pig ileum the studies confirm the lack of potency of δ -opioid ligands using both putative δ_1 preferring and putative δ_2 preferring opioid ligands. The δ -opioid ligands do have agonist properties but only at high concentrations that suggest an action *via* μ -opioid receptors in this tissue. The lack of antagonism of the μ -opioid agonists in the presence of δ -opioid ligands confirms the very low affinity of δ -opioid ligands for μ -opioid receptors. Any δ -opioid receptors present in the tissue are also unable to modulate the actions of μ -opioids since with either DPDPE or Delt II, at doses well in excess of these expected to occupy fully δ -opioid receptors, no changes in the μ -opioid agonist concentration-response curves were observed.

In conclusion the results indicate a lack of interaction between μ - and δ -opioid agonists at any level in the pathway from receptor to effector in either the mouse vas deferens or the guinea-pig ileum myenteric plexus-longitudinal muscle. Although the range of ligands studied was not exhaustive, these results lend support to the proposal (Shimohigashi *et al.*, 1987; Vaughn *et al.*, 1990; Portoghesi *et al.*, 1992) that differences exist between types of opioid receptors, or opioid systems, in the periphery and those involved centrally in antinociception (Vaughn *et al.*, 1979; Lee *et al.*, 1980; Jiang *et al.*, 1990), inhibition of gut propulsion (Heyman, 1987), volume-induced contractions of the bladder (Sheldon *et al.*, 1989) and antitussive activity (Kamei *et al.*, 1993).

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