Br. J. Pharmac. (1985), **85**, 717–726

Central δ-opioid receptor interactions and the inhibition of reflex urinary bladder contractions in the rat

A. Dray, L. Nunan & W. Wire

Department of Pharmacology, University of Arizona, Health Sciences Center, Tuscon, AZ 85724, U.S.A.

1 The *in vivo* effects of a number of opioid agonists and antagonists were studied on the spontaneous reflex contractions of the urinary bladder recorded isometrically in the rat anesthetized with urethane. All substances were administered into the central nervous system by the intracereboventricular (i.c.v.) or spinal intrathecal (i.t.) route.

2 The conformationally restricted enkephalin analogues [2-D-penicillamine, 5-L-cysteine] enkephalin (DPLCE), [2-D-penicillamine, 5-L-penicillamine] enkephalin (DPLPE) and [2-D-penicillamine, 5-D-penicillamine] enkephalin (DPDPE) produced dose-related inhibition of reflex bladder contractions when administered by the i.c.v. or i.t. route.

3 Both the novel δ-opioid receptor antagonist ICI 154,129 (200–600 μg) [N,N-bisallyl-Tyr-Gly-Gly-ψ-(CH_2S)-Phe-Leu-OH) and ICI 174,864 (1–3 μg) [N,N-diallyl-Tyr-Aib-Aib-Phe-Leu-OH: Aib = α-aminoisobutyric acid] attenuated or abolished the effects of DPLCE, DPLPE and DPDPE when administered by the i.c.v. or i.t. route. The antagonism observed was selective since the equipotent inhibition produced by the μ-opioid receptor agonist [D-Ala^2, Me-Phe^4, $Gly(ol)^5$] enkephalin (DAGO) was unaffected. Overall, ICI 154,129 was considerably weaker than ICI 174,864 and both antagonists inhibited bladder activity at doses higher than those required to demonstrate δ-receptor antagonism.

4 Further studies of the agonistic effect of ICI 174,864 showed that it was insensitive to low doses of naloxone (2 μg, i.c.v. or i.t.) but could be abolished by higher (10–15 μg) doses of naloxone. These observations suggested that the agonistic effect of ICI 174,864 was not mediated by μ-opioid receptor.

5 β-Endorphin (0.2–1.0 μg, i.c.v.) inhibited bladder contractions but following recovery from this effect, appeared to prevent the expression of δ-receptor antagonism by ICI 174,864. In addition a previously subthreshold dose of ICI 174,864 now exhibited marked agonistic activity. The inhibitory effect of a submaximal dose of DPDPE was also potentiated by β-endorphin under these circumstances.

6 These observations suggest that supra-spinal and spinal δ-opioid receptors are involved in the opioid-mediated inhibition of reflex bladder contractions in the rat. Moreover β-endorphin may be important in regulating central δ-opioid receptors.

Introduction

The effects of opioids on the central nervous system appear to be mediated through multiple populations of opioid receptors. The major receptor subtypes have been designated as μ, δ, and κ based on the selectivity of opioid agonists and antagonists in several bioassay systems (Martin *et al.*, 1976; Lord *et al.*, 1977; Paterson *et al.*, 1983).

Thus specific opioid-mediated change in physiological function appears to involve different receptors or receptor subtypes (Ling & Pasternak, 1983; Pasternak *et al.*, 1983; Porreca & Burks, 1983; Porreca *et al.*, 1984). In particular morphine and other opioids inhibit reflex contractions of the urinary bladder (Dray & Metsch, 1984a; Hisamitsu & de Groat, 1984; Jubelin *et al.*, 1984). This action appears to involve the activation of μ and δ-opioid receptors at separate supraspinal and spinal sites (Dray & Metsch, 1984a, b, c; Hisamitsu & de Groat, 1984).

To characterize further the central opioid receptors involved in the inhibition of bladder contractions, we have used a number of recently introduced (Mosberg *et al.*, 1983a, b) *bis*-penicillamine enkephalin analogues reported to be highly selective for δ-opioid receptors (Corbett *et al.*, 1983; Mosberg *et al.*, 1983a, b). In addition we have used the novel δ-opioid receptor antagonists ICI 154,129 [N,N-bisallyl-Tyr-

Gly-Gly-ψ-(CH_2S)-Phe-Leu-OH] and ICI 174,864 [N,N-diallyl-Tyr-Aib-Aib-Phe-Leu-OH: Aib = α-aminoisobutyric acid] (Gormley *et al.*, 1982; Shaw *et al.*, 1982; Cotton *et al.*, 1984) confirming the *in vivo* δ-receptor selectivity of the latter compound (Dray & Nunan, 1984). Furthermore we have observed that the endogenous opioid, β-endorphin, produced prolonged changes in the activity of central δ-opioid receptors.

Methods

Female Sprague Dawley rats (200–220g, Hilltop Laboratories, Pennsylvania) were anaesthetized with urethane (1.2 g kg^{-1}, i.p.) throughout the experiment. Body temperature was maintained at 37°C by means of a warm water blanket. The urinary bladder was catheterized via the urethra using PE50 polyethylene tubing. The intravesicular pressure was measured with a physiological pressure transducer and displayed continuously on a chart recorder. The bladder was filled with warm saline via the recording catheter until spontaneous contractions occurred as a result of central reflex activity (intravesicular pressure 5–13 cm of water). Contractions were then recorded isometrically and occurred rhythmically and reproducibly for many hours. Substances were administered into the CNS at supraspinal and spinal sites. Intracerebroventricular (i.c.v.) administrations were made into a lateral ventricle via a burr hole in the skull (coordinates 2 mm posterior to Bregma, 2 mm lateral to the midline and 4 mm deep from the skull surface). The animal's head was fixed in the stereotaxic apparatus throughout this procedure and the Hamilton syringe (26 gauge needle), which was used for microinjections, was held in a micromanipulator. Spinal intrathecal (i.t.) injections were made between the intravertebral space (vertebra L_2–L_5, usually between L_3–L_4) with the microsyringe hand-held during the procedure. All substances were administered in a volume of 1–4 μl. Microinjections of physiological saline were used as a control. These injections rarely affected bladder activity (See Figure 5).

For threshold (greater than 50% change in contraction amplitude and frequency) and potency comparisons, one dose of each substance (δ-receptor agonist) was tested per animal to avoid the possibility of tachyphylaxis or tolerance from repeated drug administrations. When repeated administrations were required as in the antagonist studies approximately 60 min was allowed between the recovery from the effect of one dose and administration of another. In the antagonist studies, microinjections of a *bis*-penicillamine enkephalin analogue were alternated with the selective μ-opioid receptor agonist [D-Ala^2, Me-Phe^4, Gly(ol)^5] enkephalin (DAGO) (Handa *et al.*, 1981). The opioid antagonists used in this study included naloxone and the novel δ-receptor selective compounds ICI 154,129 and ICI 174,864 (Gormley *et al.*, 1982; Shaw *et al.*, 1982; Cotton *et al.*, 1984; Dray & Nunan, 1984).

A 50–100% reduction of a submaximal response of the test agonist (*bis*-penicillamine enkephalin analogue) was regarded as a significant antagonism.

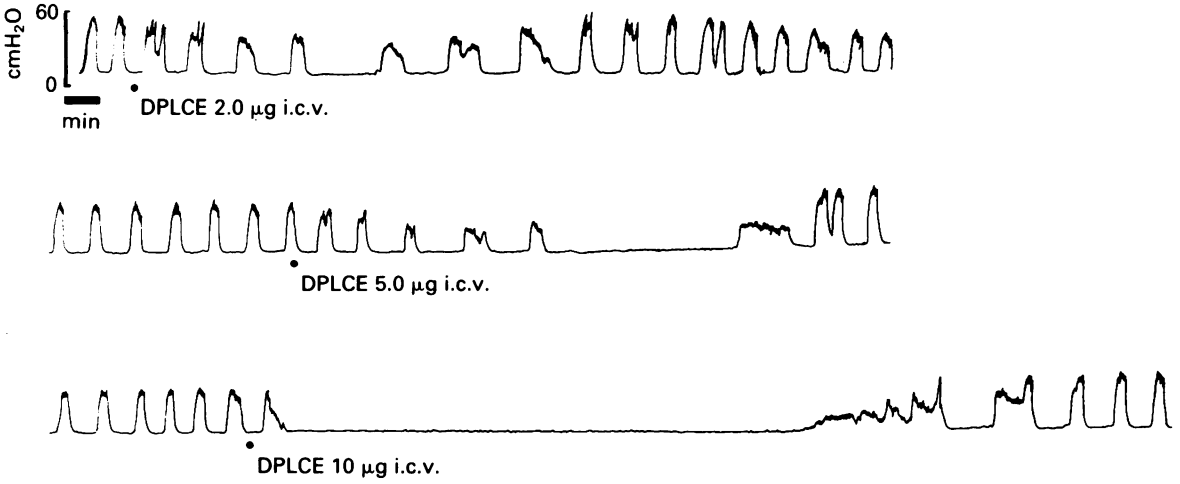

Figure 1 Inhibition of spontaneous urinary bladder contractions by [2-D-penicillamine, 5-L-cysteine] enkephalin (DPLCE). The top trace shows the effects of a threshold dose (2 μg, i.c.v.), which consists of a reduction in contraction amplitude and frequency. At a higher dose (5 μg, i.c.v.) this effect culminated in a complete cessation of bladder activity. A 10 μg, i.c.v. dose produced an abrupt cessation of bladder contraction (bottom trace). The calibration bars (cm of water and 1 min) are indicated in this and subsequent figures.

Selectivity was further assessed by the lack of effect of the δ-antagonist against inhibition of bladder contractions produced by DAGO. Usually one analogue and one antagonist was tested in an individual animal. In other studies, β-endorphin was used. The δ-receptor antagonistic effect of ICI 174,864 was first demonstrated and then the test was repeated at various periods following the administration of β-endorphin.

Unless otherwise indicated all substances were dissolved in a minimum volume of DMSO and then made up in sterile physiological saline to the required concentration. Concentrations are expressed as those of the salt. The following compounds were used in this study: [2-D-penicillamine, 5-L-cysteine]-enkephalin (DPLCE), [2-D-pencillamine, 5-L-penicillamine]-enkephalin (DPLPE), [2-D-penicillamine, 5-D-penicillamine]-enkephalin (DPDPE), (gifts from Drs Mosberg and Hruby, Department of Chemistry, University of Arizona: DPLPE was also obtained from Bachem, Bubendorf, Switzerland); β-endorphin, DAGO (Peninsula Laboratories Inc., California); naloxone hydrochloride (Endo Laboratories). ICI 154,129 and ICI 174,864 were gifts from Dr R. Cotton (ICI Pharmaceuticals Division, Macclesfield, England).

Results

Each of the *bis*-pencillamine enkephalin derivatives, DPLCE, DPLPE and DPDPE depressed reflex contractions of the urinary bladder following i.c.v. or i.t. microinjections. These effects occurred rapidly (20–90 s) and were characterized at a threshold i.c.v. or i.t. dose (DPLCE 1–2 μg, DPLPE 0.5–1.0 μg, DPDPE 0.5–1.0 μg) by a gradual reduction in the amplitude of bladder contractions and a reduction in the contraction rate (Figure 1). At suprathreshold doses this pattern either culminated in a complete cessation of bladder contractions or bladder activity ceased abruptly following the microinjection (Figure 1). The period of bladder quiescence provided an easily measured index of central drug activity and in this respect the effect of each analogue was dose-related (Table 1). The relative potency of this series of compounds appeared to be similar following i.c.v. or i.t. microinjections: DPDPE > DPLPE > DPLCE (Table 1). In addition i.t. administrations appeared somewhat more potent than the i.c.v. administrations (Table 1). This may have been due to the restricted distribution of the drug when administered by the i.t. route as determined by dye injections studied (Dray, 1985).

Bladder inhibition by submaximal doses of each δ-receptor agonist was unaffected ($n = 4$ experiments for each substance, with each route as administration) by small (1–2 μg) i.c.v. or i.t. doses of naloxone (Figure 2) which abolished the responses to equieffective doses of morphine or DAGO (Dray & Metsch, 1984b, c). On the other hand larger doses of naloxone (10–15 μg, i.c.v. or i.t.) consistently antagonized the inhibition of bladder contractions produced by submaximal doses of DPLCE, DPLPE, and DPDPE ($n = 4$–8, i.c.v. or i.t.) (Figure 3). However at these higher concentrations of naloxone a significant increase in bladder contraction frequency and sustained bladder contractions were noted (Figure 3) (Dray & Metsch, 1984b, c). It was therefore unclear whether the

Table 1 Inhibition of reflex bladder contractions by intracerebroventricular or spinal intrathecal enkephalin analogues [2-D-penicillamine, 5-L-cysteine] enkephalin (DPLCE), [2-D-penicillamine, 5-L-penicillamine] enkephalin (DPLPE) and [2-D-penicillamine, 5-D-penicillamine] enkephalin (DPDPE).

Peptide	*Intracerebroventricular* (i.c.v.) *Dose*		*Time* (min) ± s.d.	*Intrathecal* (i.t.) *Dose*		*Time* (min) ± s.d.
	μg	nmol		μg	nmol	
DPLCE	10	16	15 ± 5	5	8	22 ± 7
	20	32	25 ± 6	10	16	45 ± 8
	40	64	48 ± 5	20	32	65 ± 14
DPLPE	5	8	18 ± 4	2	4	12 ± 4
	10	16	31 ± 7	5	78	27 ± 9
	20	32	46 ± 7	10	16	56 ± 12
DPDPE	2.5	4	24 ± 6	5	8	42 ± 6
	5	8	37 ± 9	10	16	63 ± 10
	10	16	57 ± 4	20	32	94 ± 13

Doses are expressed in μg and nmol per rat. Inhibition of bladder contractions is expressed as the total time (min), for complete suppression of isometric contractions. $n = 6$–8 animals for each determination.

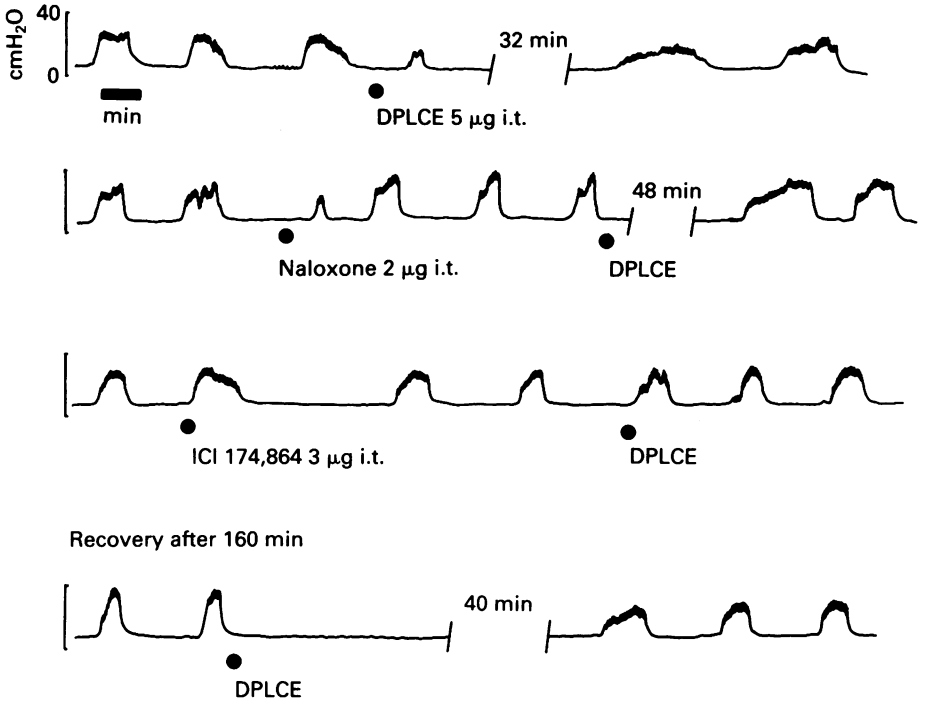

Figure 2 Antagonism of the effect of [2-D-penicillamine, 5-L-cysteine] enkephalin (DPLCE) by ICI 174,864 but not by naloxone administered intrathecally (i.t.). The top trace shows a control inhibition of reflex bladder contractions by DPLCE (5 μg, i.t.). Subsequent administrations of naloxone, (2 μg, i.t.) did not significantly affect bladder contraction frequency and did not change the response to DPLCE (5 μg, i.t.). On the other hand the i.t. administration of ICI 174,864 (3 μg, i.t.) did not change bladder contraction frequency but completely blocked the inhibitory effect of DPLCE. The effect of DPLCE returned some 160 min after ICI 174,864 (bottom trace). All traces are continuous and obtained from the same animal.

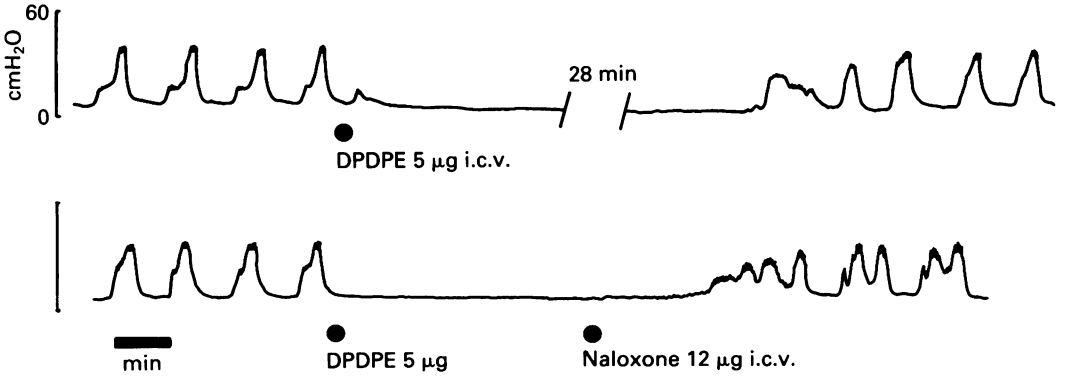

Figure 3 Antagonism of the inhibitory action [2-D-penicillamine, 5-D-penicillamine] enkephalin (DPDPE) by a high i.c.v. dose of naloxone. The cessation of bladder contractions by DPDPE (5 μg, i.c.v.) was not antagonized by a low dose (2 μg, i.c.v.) of naloxone (not shown) but was reversed by a higher doses (12 μg, i.c.v.) of naloxone. Note also the increase in bladder activity produced by this high dose of naloxone was manifested by the occurrence of multiple and more rapid bladder contractions. Approximately 120 min elapsed between the first naloxone dose and the administration of another.

effects of these higher doses of naloxone were due to a pharmacological antagonism or a functional antagonism.

Further studies using ICI 154,129 and ICI 174,864 showed that these compounds produce selective δ-receptor antagonism. Thus ICI 154,129 (200–600 μg) antagonized the effects of DPLCE (3 of 3 experiments, i.c.v.), DPLPE (4 of 4 experiments, i.c.v.) and DPDPE (11 of 12 experiments, i.c.v.) without affecting the responses to DAGO. However, it was notable that with higher doses of ICI 154,129 direct dose-related agonistic effects were also observed (6 of 12 experiments) (inhibition of bladder activity 400 μg = 12 ± 4 min, $n = 3$; 600 μg = 32 ± 12 min, $n = 3$). By contrast smaller i.c.v. or i.t. doses of ICI 174,864 (1–3 μg) produced selective antagonism of DPLCE (4 of 4 experiments, each route) DPLPE (4 of 4 experiments, each route) and DPDPE (16 of 16 experiments, i.c.v.; 6 of 6 experiments, i.t.) without changing the equipotent actions of DAGO (Figure 4)

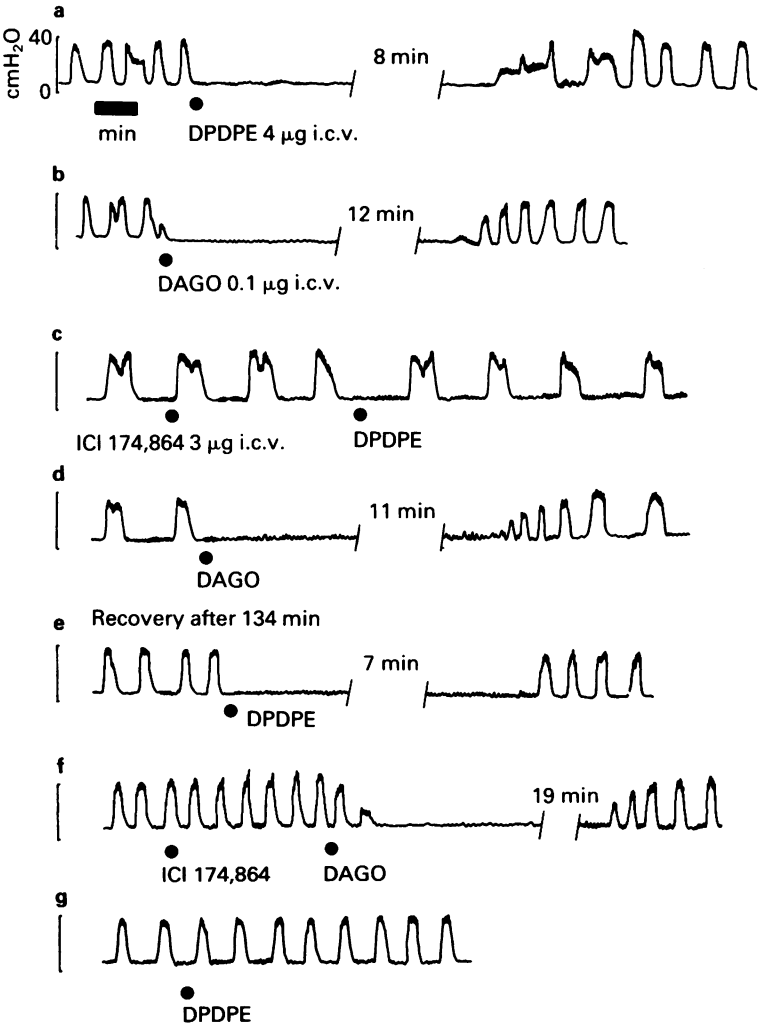

Figure 4 Selective antagonism of the effect of the δ-receptor agonist [2-D-penicillamine, 5-D-penicillamine] enkephalin (DPDPE) but not of the μ-receptor agonist [D-Ala^2, Me-Phe^4, Gly(ol)^5] enkephalin (DAGO) by ICI 174,864. Traces (a) and (b) show the control inhibitory responses produced by DPDPE (4 μg, i.c.v.) and DAGO (0.1 μg, i.c.v.). Shortly after the i.c.v. administration of ICI 176,864 (3 μg, i.c.v.) the effect of DPDPE was completely blocked whereas that of DAGO was virtually unchanged (c and d). Moreover ICI 174,864 produced no obvious changes in contraction frequency. Recovery of DPDPE from the antagonistic effect of ICI 174,864 was observed some 134 min later (e). A repeated administration of ICI 174,864 with a reversal of the order of agonist administration, also selectively abolished the effect of DPDPE (f and g). All traces are continuous and were obtained from the same animal.

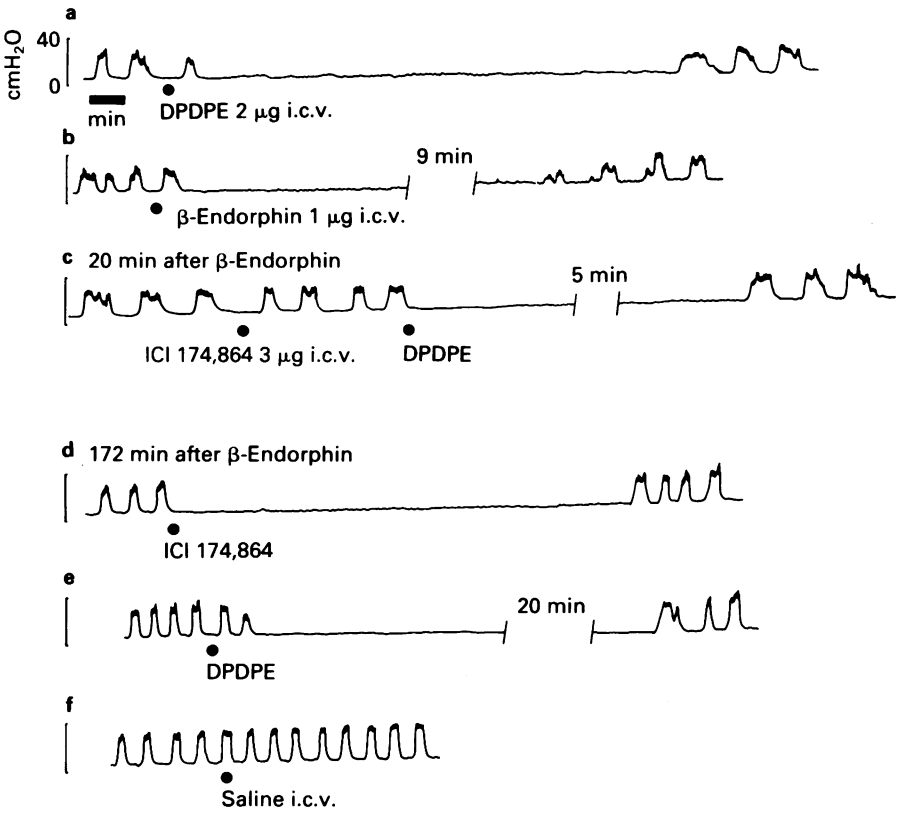

Figure 5 β-Endorphin prevented the δ-antagonistic effect of ICI 174,864, revealed the agonist action of ICI 174,864 and potentiated bladder inhibition by [2-D-penicillamine, 5-D-penicillamine] enkephalin (DPDPE). Traces (a) and (b) show the inhibition of reflex bladder contractions produced by DPDPE (2 μg, i.c.v.) and by β-endorphin (1 μg, i.c.v.). When the δ-receptor antagonist ICI 174,864 (3 μg, i.c.v.) was subsequently tested 20 min after recovery from the β-endorphin effect it did not block the effect of DPDPE (c). The middle and bottom traces show that when the administration of a previously subthreshold dose of ICI 174,864 (3 μg, i.c.v.) was repeated (172 min after β-endorphin) (d) it now produced a significant inhibition of reflex bladder contractions (approximately 13 min). In addition the effects of DPDPE, tested 60 min later (e), was markedly prolonged (approximately 200% of control). All traces are continuous and are taken from the same animal. Trace (f) shows that saline administration (3 μl, i.c.v.) did not affect bladder activity.

or morphine (1 μg, 3 of 3 experiments, i.c.v.; 2 of 2 experiments, i.t.) and without producing changes in spontaneous bladder contraction frequency (Figure 2, 4). With lower doses of ICI 174,864 (1 μg, i.c.v., i.t.) antagonism was observed less consistently (2 of 6 experiments, i.c.v.; 3 of 6 experiments, i.t.).

Complete recovery of the effects of one or other of the δ-receptor agonists occurred some 96 to 240 min following the microinjection of ICI 154,129 or ICI 174,864 (Figure 3, 4). In a number of experiments (9 experiments, i.c.v.; 4 experiments, i.t.) the sequence of δ and μ-agonist administration was reversed following complete recovery from the effects of ICI 174,864. The repeated administration of ICI 174,864 once again selectively blocked the effect of DPDPE and not DAGO (Figure 4).

At doses higher than those required to demonstrate selective δ-receptor antagonism, ICI 174,864 itself consistently exhibited agonistic activity and suppressed bladder contractions in a dose-related manner (5 μg = 13 ± 3 min; 10 μg = 29 ± 6 min; 15 μg = 49 ± 16 min, $n = 5$ for each i.c.v. determination). Delta receptor antagonism could however still be demonstrated following recovery from the agonistic effect of ICI 174,864 (6 of 6 experiments, i.c.v. using DPDPE). The depressant effect of ICI 174,864 (10 μg, i.c.v. or i.t.) was not abolished, but could be attenuated (4 of 8 experiments, i.c.v.; 2 of 6 experiments, i.t.) by a small

dose (2 μg, i.c.v. or i.t.) of naloxone. Higher doses of naloxone (10 μg, $n = 4$ for each route of administration) abolished the inhibitory effect of ICI 174,864. As before it was unclear whether this was a pharmacological or functional antagonism by naloxone.

In the final series of experiments involving ICI 174,864, β-endorphin was used as an opioid agonist. Thus β-endorphin produced dose-related (0.5 μg = 10 ± 4 min, $n = 5$; 1 μg = 27 ± 10 min, $n = 7$, i.c.v.) inhibition of bladder contractions as described previously (Dray *et al.*, 1984). However when ICI 174,864 was retested for δ-receptor antagonistic activity, following (20–60 min) either a subthreshold dose of β-endorphin (0.1–0.2 μg, i.c.v., $n = 4$) or more commonly (10 experiments) after the agonist effect of β-endorphin had been demonstrated, it no longer behaved as an antagonist (14 of 14 experiments) (Figure 5). Additionally in other experiments (7 of 7 experiments) where antagonism toward DPDPE and not DAGO had been demonstrated, a repeated test with ICI 174,864 failed to exhibit antagonistic activity following the prior administration of β-endorphin (1 μg, i.c.v.).

When ICI 174,864 or DPDPE were tested from 20 to 68 min after a β-endorphin administration no difference in their effect on bladder contractions was noted (9 of 9 experiments, Figure 5). However when these agents were retested at later periods (90 to 278 min) at the same dose, ICI 174,864 was observed (14 of 17 experiments, i.c.v.) to produce agonistic effects (3 μg, i.c.v. = 16 ± 6 min, $n = 14$) (Figure 5), while the inhibition of bladder contractions by DPDPE appeared potentiated (9 of 9 experiments;control i.c.v. DPDPE = 13 ± 2 min: post β-endorphin = 25 ± 6 min, $n = 9$). When saline microinjections were used in place of β-endorphin no significant changes in the activity of ICI 174,864 or DPDPE were noted (4 of 4 experiments for each substance, i.c.v.).

Discussion

Previous studies have demonstrated that opioids administered systemically or by direct microinjection into different regions of the CNS inhibited reflex contractions of the rat (Brent *et al.*, 1983, Dray & Metsch, 1984a, b, c) and cat (Hisamitsu & de Groat, 1984; Jubelin *et al.*, 1984) urinary bladder. Moreover these effects were shown to be mediated supraspinally and spinally by activation of μ-opioid receptors and probably δ-receptors but not by κ-receptors (Brent *et al.*, 1983; Dray & Metsch, 1984a, b, c; Hisamitsu & DeGroat, 1984). In the present experiments a series of conformationally constrained *bis*-penicillamine enkephalin analogues (Mosberg *et al.*, 1983a, b), shown to exhibit unique selectivity for the δ-opioid receptor (Corbett *et al.*, 1983; Mosberg *et al.*, 1983a, b) have been used to explore further the involvement of opioid receptor subtypes. These compounds produced dose-related inhibition of reflex bladder contractions when administered into a lateral ventricle or into sacral regions of the spinal cord. These findings therefore confirm previous conclusions, which were based on observations with less selective ligands, that central δ-opioid receptors were involved in opioid mediated inhibition of reflex bladder contractions.

It is not clear at present whether exogenously administered opioid receptor ligands produce their central effects on bladder activity by mimicking endogenous processes. However an interaction with a supraspinal micturition centre in the brain stem (Satoh *et al.*, 1978) and an action on spinal sacral parasympathetic motor outflow to the bladder have been postulated as possible sites for opioid-mediated changes in bladder activity (Roppolo *et al.*, 1983; Hisamitsu & DeGroat, 1984; Dray & Metsch, 1984a, b, c).

In the second part of our study the novel δ-opioid receptor antagonists ICI 154,129 and ICI 174,864 were used to confirm the selectivity of the interactions observed and to characterize further central δ-opioid receptor activity. ICI 154,129 has been previously shown to be a weak but relatively selective δ-receptor antagonist with some degree of central μ-agonistic properties at higher doses (Gormley *et al.*, 1982; Shaw *et al.*, 1982; Vinaky *et al.*, 1983; D'Amato & Holaday, 1984; Tortella *et al.*, 1984). The present observations broadly confirm these previous conclusions. Thus ICI 154,129 selectively abolished the effects of each of the δ-receptor ligands. Additionally, higher doses of ICI 154,129 produced agonistic activity, though the mechanism of this effect was not determined. In contrast, relatively low doses of ICI 174,864, a δ-receptor antagonist *in vitro* (Cotton *et al.*, 1984) and in our preliminary *in vivo* study (Dray & Nunan, 1984), selectively attenuated or abolished the effect of each of the novel δ-receptor ligands. However ICI 174,864 like ICI 154,129 also produced agonistic effects at higher doses than those required for selective δ-receptor antagonism. These agonistic effects were not reversed by small doses of naloxone, which selectively antagonized bladder inhibition produced by μ-receptor ligands (Dray & Metsch, 1984b, c), but could be reversed by larger doses of naloxone. However, at the higher doses naloxone itself produced an increase in frequency of bladder contractions. Therefore it was unclear whether the reversal of the agonistic effect of ICI 174,864 were due to a pharmacological or functional antagonism by naloxone.

The final series of experiments revealed that β-endorphin produced long-lasting changes in central δ-receptor activity. Thus following β-endorphin administration, ICI 174,864 no longer behaved as an antagonist, but on the contrary, exhibited marked

agonistic activity at previously subthreshold doses. In addition the effects of the δ-ligand DPDPE but not the μ-ligand DAGO were potentiated. These observations suggested that β-endorphin produced a selective change in central δ-opioid receptors rendering their interaction with agonists to be more efficacious but that to antagonists to be less efficacious.

The prolonged changes induced in the activity of ICI 174,864 and in DPDPE occurred after the direct agonistic effect of β-endorphin was no longer detectable. Indeed similar effects were observed at doses of β-endorphin which were insufficient to inhibit bladder activity. It would seem that the effects observed were likely to be due to β-endorphin and not a fragment derived from *in vivo* metabolism since this peptide is processed relatively slowly by central protease activity (Davis *et al.*, 1983). In addition β-endorphin is highly lipophilic (Meek, 1980; Wilson *et al.*, 1981) and may thus remain bound in a biologically active form for a considerable period.

It is not clear at present how the effects of β-endorphin were mediated. A number of *in vitro* and *in vivo* studies have demonstrated that β-endorphin may interact in a complex manner with a variety opioid receptor subtypes (μ, δ, μ_1, ε) (Ferrara *et al.*, 1979; Akil *et al.*, 1980; Wuster *et al.*, 1980; Schulz *et al.*, 1981; Ferrara & Li, 1980; Hazum *et al.*, 1979; Ling & Pasternak, 1983; Goodman *et al.*, 1983; Ho *et al.*, 1983; Lin-Shiau *et al.*, 1983; Houghten *et al.*, 1984; Dray *et al.*, 1984). These interactions may or may not be related to the effects of β-endorphin on δ-receptor activity. However, it is attractive to speculate that the interaction of β-endorphin with a δ-receptor complex was preceded by an activation of a closely associated macromolecule. In support of this, β-endorphin and other naturally occurring opioid peptides such as dynorphin 1–13 and enkephalins have been postulated to bind to different sites on a common receptor complex (Lee & Smith, 1980; 1984; Smith *et al.*, 1983). Such interactions may change allosterically the 'antagonistic' and 'agonistic' conformation of the δ-receptor. In this respect it is also noteworthy that conformational changes in the opioid receptor complex have been noted by others. Thus μ and δ-receptor interconversion (Bowen *et al.*, 1981) and interactions have been reported (Lee & Smith, 1980; Rothman & Westfall, 1982; Vaught *et al.*, 1982; Smith *et al.*, 1983; Holaday, *et al.*, 1983, D'Amato & Holaday, 1984). Additionally κ-ligands (dynorphin 1–13) produced complex interactions with μ- (morphine, naloxone) and δ-receptors (ICI 174,864) (Friedman *et al.*, 1981; Tulunay *et al.*, 1981; Holaday *et al.*, 1984; Long *et al.*, 1984). In this latter regard there are direct similarities with the present findings. Thus pretreatment with dynorphin 1–13 prevented the therapeutic effect of ICI 174,864 on endotoxin-induced hypotension (Long *et al.*, 1984). Possibly a common macromolecule may be involved in a number of chemically regulated activities.

In conclusion, several uniquely selective δ-opioid receptor ligands (DPLCE, DPLPE and DPDPE) have been shown to produce dose-related inhibition of reflex bladder contractions when administered by the i.c.v. or spinal intrathecal route. Moreover the effects of these agonists at either site in the central nervous system were selectively reversed by the novel δ-opioid receptor antagonists ICI 154,129 and ICI 174,864. These antagonists however, also exhibited agonistic activity at higher doses. These properties might limit their usefulness as research tools. In the case of ICI 174,864 the agonistic effect did not appear to be mediated through μ-opioid activity. Finally, prior administration of β-endorphin both prevented the δ-receptor antagonistic effect of ICI 174,864 and revealed its agonistic activity. In addition the inhibition of bladder activity by DPDPE was potentiated. These data thus support the involvement of supraspinal and spinal δ-opioid receptors in the opioid-mediated inhibition of reflex bladder activity in the rat. They also indicate that β-endorphin may have important regulatory effects on central δ-opioid receptor activity.

References

AKIL, H., HEWLETT, A.A., BARCHAS, J.D. & LI, C.H. (1980). Binding of [^{3}H]-β-endorphin to rat-brain membranes: characterization of opiate properties and interactions with ACTH. *Eur. J. Pharmac.*, **64**, 1–8.

BOWEN, W.D., GENTLEMAN, S., HERKENHAM, M. & PERT, C. (1981). Interconverting mu and delta forms of the opiate receptor in rat striatal patches. *Proc. natn. Acad. Sci. U.S.A.*, **78**, 4818–4822.

BRENT, C.R., HARTY, G. & YAKSH, T.L. (1983). The effects of spinal opiates on micturition in unanesthetized animals. *Soc. Neuroscience*, **9**, 743.

CORBETT, A.D., GILLAN, M.G.C., KOSTERLITZ, H.W., McKNIGHT, A.T. & PATERSON, S.J. (1983). Tyr-D-Pen-Gly-Phe-L-Pen and Tyr-D-Pen Gly-Phe-D-Pen are selective ligands for the δ-binding site. *Br. J. Pharmac.*, **80**, 669P.

COTTON, R., GILES, M.G., MILLER, L., SHAW, J.S. & TIMMS, D. (1984). ICI 174,864: a highly selective antagonist for the opioid δ-receptor. *Eur. J. Pharmac.*, **97**, 331–332.

D'AMATO, R. & HOLADAY, J.W. (1984). Multiple opioid receptors in endotoxic shock: evidence for involvement and μ-δ interactions *in vivo*. *Proc. natn. Acad. Sci. U.S.A.*, **81**, 2898–2901.

DAVIS, T.P., SCHOEMAKER, H., CULLING, A.J. & YAMAMURA, H.I. (1983). Isolation and characterization of *in vivo* central β-endorphin metabolism in schizophrenia. *Proc. West. Pharmac. Soc.*, **26**, 89–94.

DRAY, A. (1985). The rat urinary bladder, a novel preparation for investigating central opioid activity *in vivo*. *J. Pharmac. Meth.* (press).

DRAY, A. & METSCH, R. (1984a). Morphine and the centrally mediated inhibition of urinary bladder motility in the rat. *Brain Research*, **297**, 191–195.

DRAY, A. & METSCH, R. (1984b). Opioids and central inhibition of urinary bladder motility. *Eur. J. Pharmac.*, **98**, 155–156.

DRAY, A. & METSCH, R. (1984c). Inhibition of urinary bladder contractions by a spinal action of morphine and other opioids. *J. Pharmac. exp. Ther.*, **231**, 254–260.

DRAY, A., METSCH, R. & DAVIS, T.P. (1984). Endorphins and the central inhibition of urinary bladder motility. *Peptides*, **5**, 645–647.

DRAY, A. & NUNAN, L. (1984). Selective δ-opioid receptor antagonism by ICI 174,864 in the central nervous system. *Peptides*, **5**, 1015–1016.

FERRARA, P., HOUGHTEN, R. & LI, C.H. (1979). β-Endorphin: characteristics of binding sites in the rat brain. *Biochem. biophys. Res. Commun.*, **89**, 786–792.

FERRARA, P. & LI, C.H. (1980). β-Endorphin: characteristics of binding sites in rabbit spinal cord. *Proc. natn. Acad. Sci. U.S.A.*, **77**, 5746–5748

FRIEDMAN, H.J., JEN, M.F., CHANG, J.K., LEE, N.M. & LOH, H.H. (1981). Dynorphin: a possible modulatory peptide on morphine or β-endorphin analgesia in mouse. *Eur. J. Pharmac.*, **69**, 357–360.

GOODMAN, R.R., HOUGHTEN, R.A. & PASTERNAK, G.W. (1983). Autoradiography of ^{3}H-β-endorphin binding in brain. *Brain Res.*, **288**, 334–337.

GORMLEY, J.J., MORLEY, J.S., PRIESTLEY, T., SHAW, J.S., TURNBULL, M.J. & WHEELER, H. (1982). *In vivo* evaluation of the opiate delta receptor antagonist ICI 154,129. *Life Sci.*, **31**, 1263–1266.

HANDA, B.K., LANE, A.C., LORD, J.A.H., MORGAN, B.A., RANCE, M.J. & SMITH, C.F.C. (1981). Analogs of beta-LPH_{61-64} possessing selective agonists activity of μ-opiate receptors. *Eur. J. Pharmac.*, **70**, 531–540.

HAZUM, E., CHANG, K.J. & CUATRECASAS, P. (1979). Interaction of iodinated human (D-Ala^2) β-endorphin with opioid receptors. *J. biol. Chem.*, **251**, 1765–1767.

HISAMITSU, T. & DEGROAT, W.C. (1984). The inhibitory effect of opioid peptides and morphine applied intrathecally and intracerebroventricularly on the micturition reflex in the cat. *Brain Res.*, **298**, 51–65.

HO, C.L., HAMMONDS, R.G. & LI, C.H. (1983). β-Endorphin: characteristics of binding sites in rabbit cerebellar and brain membranes. *Biochem. biophys, Res. Commun.*, **111**, 1096–1104.

HOLADAY, J.W., KENNER, J.R., GLATT, C.E., & LONG, J.B. (1984). Dynorphin: cardiovascular consequences of opioid receptor interactions in normal and endotoxemic rats. *Proc. West. Pharmac. Soc.*, **27**, 429–433.

HOLADAY, J.W., PASTERNAK, G.W., D'AMATO, R.J., RUVIO, B.A. & FADEN, A.I. (1983). Naloxazone lacks therapeutic effects in endotoxic shock yet blocks the effects of naloxone. *Eur. J. Pharmac.*, **89**, 293–296.

HOUGHTEN, R.A., JOHNSON, N. & PASTERNAK, G.W. (1984). [^{3}H]-β-endorphin binding in rat brain. *J. Neuroscience*, **4**, 2460–2465.

JUBELIN, B., GALEANO, C., LADOUCEUR, D., LEMAIRE, S. & ELHILALI, M.M. (1984). Effect of enkephalin on the micturition cycle of the cat. *Life Sci.*, **34**, 2015–2027.

LEE, N.M. & SMITH, A.P. (1980). A protein-lipid model of the opiate receptor. *Life Sci.*, **26**, 1459–1464.

LEE, N.M. & SMITH, A.P. (1984). Possible regulatory function of dynorphin and its clinical implications. *Trends Pharmac. Sci.*, **5**, 108–110.

LING, G.S.F., MACLEOD, J.M., LEE, S., LOCKHART, S.H. & PASTERNAK, G.W. (1984). Separation of morhpine analgesia from physical dependence. *Science*, **226**, 462–464.

LING, G.S.F. & PASTERNAK, G.W. (1982). Morphine catalepsy in the rat: Involvement of mu_1 (high affinity) opioid binding sites. *Neurosci. Letts.*, **32**, 193–196.

LING, G.S.F. & PASTERNAK, G.W. (1983). Spinal and supraspinal opioid analgesia in the mouse: role of subpopulations of opioid binding sites. *Brain Research*, **271**, 152–156.

LIN-SHIAU, S.Y., HAMMONDS, R.G. & LI, C.H. (1983). β-Endorphin: characteristics of binding sites in the mouse brain. *Euro. J. Pharmac.*, **87**, 297–300.

LONG, J.B., RUVIO, B.A., GLATT, C.E. & HOLADAY, J.W. (1984). ICI 174,864, a putative delta opioid antagonist, reverses endotoxemic hypotension: pretreatment with dynorphin 1–13, a κ-agonist, blocks this action. *Neuropeptides*, in press.

LORD, J.A.H., WATERFIELD, A.A., HUGHES, J. & KOSTERLITZ, H.W. (1977). Endogenous opioid peptides: multiple agonists and receptors. *Nature (Lond)*, **267**, 295–299.

MARTIN, W.R., EADES, C.G., THOMPSON, J.A., HUPPLER, R.E. & GILBERT, P.E. (1976). The effects of morphine- and nalorphine-like drugs in nondependent and morphine-dependent chronic spinal dog. *J. Pharmac. exp. Ther.*, **197**, 517–532.

MEEK, J.L. (1980). Prediction of peptide retention times in high-pressure liquid chromatography as the basis of amino acid composition. *Proc. natn. Acad. Sci. U.S.A.*, **77**, 1632–1636.

MOSBERG, H.I., HURST, R., HRUBY, V.J., GALLIGAN, J.J., BURKS, T.F., GEE, K. & YAMAURA, H.I. (1983a). Conformationally constrained cyclic enkephalin analogs with pronounced delta opioid receptor agonist selectivity. *Life Sci.*, **32**, 2565–2569.

MOSBERG, H.I., HURST, R., HRUBY, V.J., GEE, K., YAMAURA, H.I., GALLIGAN, J.J. & BURKS, T.P. (1983b). *Bis*-penicillamine enkephalins possess highly improved specificity towards delta opioid receptors. *Proc. natn. Acad. Sci. U.S.A.*, **80**, 5871–5874.

PASTERNAK, G.W., GINTZLER, A.R., HOUGHTEN, R.A., LING, G.S.F., GOODMAN, R.R., SPIEGEL, K., NISHIMURA, S., JOHNSON, N. & RECHT, L.D. (1983). Biochemical and pharmacological evidence for opioid receptor multiplicity in the central nervous system. *Life Sci.*, **33**, Suppl. 1, 167–173.

PATERSON, S.J., ROBSON, L.E. & KOSTERLITZ, H.W. (1983). Classification of opioid receptors. *Br. med. Bull.*, **39**, 31–36.

PORRECA, F. & BURKS, T.F. (1983). The spinal cord as a site of opioid effects on gastrointestinal transit in the mouse. *J. Pharmac. exp. Ther.*, **227**, 22–27.

PORRECA, F., MOSBERG, H.I., HURST, R., HRUBY, V.J. & BURKS, T.F. (1984). Roles of mu, delta and kappa opioid receptors in spinal and supraspinal mediation of gastrointestinal transit effects and hot-place analgesia in the mouse. *J. Pharmac. exp. Ther.*, **230**, 341–348.

ROPPOLO, J.R., BOOTH, A.M. & DEGROAT, W.C. (1983). The

effects of naloxone on the neural control of the urinary bladder of the cat. *Brain Res.*, **264**, 355–358.

ROTHMAN, R.B. & WESTFALL, T.C. (1982). Allosteric coupling between morphine and enkephalin receptors *in vitro* (1982). *Molec. Pharmac.*, **21**, 548–557.

SATOH, K., SHIMIZU, N., TOHYAMA, M. & MAEDA, T. (1978). Localization of the micturition reflex center at dorso-lateral pontine tegmentum of the rat. *Neurosci. Letts.*, **8**, 27–33.

SCHULZ, R., WUSTER, M. & HERZ, A. (1981). Pharmacological characterization of the epsilon opiate receptor. *J. Pharmac. exp. Ther.*, **216**, 786–792.

SHAW, J.S., MILLER, L., TURNBULL, M.J., GORMLEY, J.J. & MORLEY, J.S. (1982). Selective antagonists of the opiate delta-receptor. *Life Sci.*, **31**, 1259–1262.

SMITH, A.P., LEE, N.M. & LOH, H.H (1983). The multiple site β-endorphin receptor. *Trends Pharmac. Sci.*, **4**, 163–164.

TORTELLA, F.C., ROBLES, L.E., HOLADAY, J.W. & COWAN, A. (1984). ICI 154,129, a δ-opioid receptor antagonist raises seizure threshold in rats. *Eur. J. Pharmac.*, **97**, 141–144.

TULUNAY, F.C., JEN, M.-F., CHANG, J.-K., LOH, H.H. & LEE, N.M. (1981). Possible regulatory role of dynorphin on morphine- and β-endorphine-induced analgesia. *J. Pharmac. exp. Ther.*, **219**, 296–298.

VAUGHT, J.L., ROTHMAN, R.B. & WESTFALL, R.C. (1982). Mu and delta receptors: their role in analgesia and in the differential effects of opioid peptides on analgesia. *Life Sci.*, **30**, 1443–1455.

VINAYEK, R., BROWN, D.R. & MILLER, R.J. (1983). Inhibition of the antisecretory effects [D-Ala^2,D-Leu^5] enkephalin in the guinea-pig ileum by a selective δ opioid antagonist. *Eur. J. Pharmac.*, **94**, 159–161.

WILSON, K.J., HONEGGLER, A., STOTZEL, R.P. & HUGHES, G.J. (1981). The behaviour of peptides on reverse-phase supports during high-pressure liquid chromatogrpahy. *Biochem. J.*, **199**, 31–41.

WUSTER, M., SCHULZ, R. & HERZ, A. (1980). The direction of opioid agonists towards mu, delta and epsilon receptors in the vas deferens of the mouse and rat. *Life Sci.*, **27**, 163–170.

(Received January 21, 1985.
Accepted February 22, 1985.)