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Evidence for Functional δ-Opiate Receptors in the Rat Intestine

DEBRA L. HANCOCK AND IAN M. COUPAR

Unit of Addictive Drug Research, School of Pharmaceutical Pharmacology, Victorian College of Pharmacy, Monash University, 381 Royal Parade, Parkville, Victoria 3052, Australia

Abstract—The selective δ-opiate agonists D-Ser^2, Leu^5, Thr^6-enkephalin (DSLET), D-Ala^2, D-Leu^5-enkephalin and D-Pen^2, D-Pen^5-enkephalin caused inhibition of the cholinergic contraction produced by transmural stimulation of the rat isolated jejunum. Dynorphin A, which is an agonist at both κ- and δ-opioid receptors also inhibited the cholinergic contraction, as did leu- and met-enkephalin. The selective μ-receptor agonist D-Ala^2-NMe-Phe^4, Gly-ol^5-enkephalin was the least potent of all peptides tested. In general, the order of potency of the peptides was similar to that reported for the δ-receptor-rich mouse vas deferens with potency values similar to those recorded previously for the hamster vas deferens. The selective δ-opioid antagonist naltrindole caused parallel shifts to the concentration-effect curve to DSLET giving a pA_2 value of 10·15. The results indicate that the previously identified δ-binding sites in the rat jejunum may correspond to functional δ-opiate receptors involved in attenuating acetylcholine release.

Numerous studies have been performed using the guinea-pig ileum to investigate the action of opiates on intestinal function (Paton 1957; Collier et al 1981). However, the effects and mechanisms of opiate action on the intestine is known to be species dependent (Weinstock 1971). For instance, morphine does not inhibit low-frequency cholinergic transmission in the ileum of the mouse (Smith et al 1988) or the rabbit (Oka 1981) as it does in the guinea-pig (Paton 1957).

Classical bioassays such as the guinea-pig ileum and the mouse vas deferens have been used to screen novel agonists and antagonists for selectivity. These assays exploit the presence of μ- and κ-opiate receptors in the guinea-pig ileum and δ-receptors in the mouse vas deferens as a means of measuring selectivity (Hutchinson et al 1975; Lord et al 1977; Chavkin et al 1982). The use of these tissues, coupled with homogenate binding, has resulted in the subsequent development of highly selective opiate agonists and the characterization of opiate receptors in other tissues. For example, δ- and κ-opiate receptors involved in attenuating acetylcholine release have been identified in the ileum of the rabbit and the mouse (Oka 1981; Smith et al 1988). Surprisingly, the rat has not been studied to determine the functional opiate receptor subtype present in the intestine. It is likely that opiate receptors are associated with the modulation of neuromuscular transmission in the intestine of this commonly-used laboratory animal for the following reasons. It is known that the rat small intestine contains enkephalinergic nerves which are localized to the myenteric plexus and circular muscle layer (Schultzberg et al 1980; Ekbland et al 1991). Further, autoradiographic studies have revealed both μ- and δ-binding sites (Dashwood et al 1985; Nishimura et al 1986). The μ-sites which are localized predominantly to the villi and submucous plexus could represent the receptors at which morphine and other μ-agonists inhibit intestinal fluid secretion (Coupar 1983). The δ-binding sites, as defined by [^{3}H]D-Ala^2, D-Leu^5-enkephalin (DADLE), are also localized to the mucosa and submucous plexus (Dashwood et al 1985; Nishimura et al 1986). Our preliminary studies have shown that DADLE inhibits the cholinergic contractile response to transmural stimulation of the rat isolated small intestine (Coupar & De Luca 1991). The aim of the present study was to characterize further the DADLE-sensitive receptor using a range of opioid peptide agonists with varying selectivities for opiate receptors and a selective antagonist.

Correspondence: I. M. Coupar, Unit of Addictive Drug Research, School of Pharmaceutical Pharmacology, Victorian College of Pharmacy, Monash University, 381 Royal Parade, Parkville, Victoria 3052, Australia.

Materials and Methods

Tissue preparation

Hooded Wistar rats, 250–320 g, of either sex were stunned by a blow to the head and killed by exsanguination. Usually four adjacent segments (2·5–3 cm in length) of proximal jejunum were excised from each rat and mounted in 30-mL jacketed organ baths maintained at 37°C. Each segment of jejunum represents n = 1. The preparations were allowed to equilibrate for 45 min in a physiological salt solution of the following composition (mM): NaCl 118, KCl 4·7, $NaHCO_3$ 25, KH_2PO_4 1·2, $CaCl_2$ 2·5, $MgSO_4$ 1·2, D-(+)-glucose 11.

Changes in the length of the longitudinal muscle were measured under a load of 1 g with an Ugo Basile isotonic transducer connected to a Grass model 79D polygraph which amplified the responses 5- to 20-fold. Preliminary experiments showed that transmural stimulation at a frequency of 10 Hz lasting 8 s produced a submaximal contraction of the tissues compared with higher and lower frequencies also lasting the same time. Consequently, the segments were stimulated at 10 Hz for 8 s in all further experiments. The pulses were delivered from a Grass S44 stimulator through parallel platinum electrodes at a duration of 1 ms, supramaximal voltage. The 8-s trains

were delivered every 5 min and each segment was exposed to a single concentration of drug for a period of 1 min.

Drugs

The following drugs were used: D-Ala^2, D-Leu^5-enkephalin (DADLE, Bachem, Bubendorf, Switzerland), D-Ala^2,NMe-Phe^4,Gly-ol^5-enkephalin (DAGO, Auspep, Melbourne, Australia), atropine sulphate (Sigma, Castle Hill, Australia), dynorphin A 1-13 (Porcine) (Auspep), Leu^5-enkephalin (Auspep), naltrindole HCl (RBI, Natick, USA), neostigmine (Roche), Met^5-enkephalin (Auspep), D-Ser^2,Leu^5, Thr^6-enkephalin (DSLET, Auspep), D-Pen^2,D-Pen^5-enkephalin (DPDPE, Bachem), and thiorphan (RBI). All drugs were dissolved in distilled water except thiorphan which was dissolved in 0·238 M $NaHCO_3$.

Statistical analysis

The potencies of the opioid peptides are expressed as IC50 values with 95% confidence limits. Differences between means and lines were considered statistically different when $P < 0.05$.

Results

Responses to nerve stimulation

Transmural stimulation of the jejunum for 8 s produced a complex of frequency-related responses, both during and after the period of stimulation. The threshold frequency for inducing the responses was 2·5 Hz and the maximal was 20 Hz. The pulse frequency used in this series of experiments was set at 10 Hz for 8 s since it resulted in clearly quantifiable responses. These consisted of a fast contraction which was sometimes preceded by a small relaxation. After the period of stimulation a large but slow contraction followed which was often greater than the initial contraction (Fig. 1). Atropine (100 nM, 10-min incubation) blocked the contraction which occurred during the period of nerve stimulation (n = 12). In these experiments, atropine unmasked an initial relaxation but did not affect the after-contraction. Neostigmine (75 nM, 1 min incubation) increased the atropine-sensitive response to 142 ± 7% of control (n = 11). In separate experiments, matched responses to acetylcholine (100 nM, 10 s) were also potentiated by neostigmine to 144 ± 8% of control (n = 12).

Table 1. Potencies of opioid peptides in inhibiting cholinergic transmission in segments of rat jejunum.

Opioid	IC50 (nM)	(n)	95% Confidence limits
DSLET	13·46	(36)	2·44
DADLE	38·3	(27)	2·37
Dynorphin A	95·99	(19)	16·97
Met-enkephalin	101·75	(18)	1·73
Leu-enkephalin	184·11	(17)	2·88
DPDPE	246·31	(15)	3·34
DAGO	714·58	(19)	1·83

Responses of opioid peptides

It was established using DADLE that an incubation time of 1 min was sufficient to produce maximum inhibition of the cholinergic contractile responses (Fig. 1). All opioid peptides tested reduced the initial cholinergic contraction of the jejunum and, like atropine, potentiated or unmasked the initial inhibitory response without affecting the after-contraction. The most potent opioid was DSLET and the least potent DAGO (Table 1, Fig. 2).

Effect of naltrindole

Naltrindole caused parallel displacements of the concentration-effect line to DSLET at concentrations of 0·2, 2 and 20 nM (15-min incubations, Fig. 3). Schild analysis of the resultant dose ratios gave a pA_2 value of 10·15 ± 0·11 with a slope of −1·04, which was not significantly different from −1 ($P > 0.05$). The corresponding pK_B value (slope constrained to −1) was 10·22 ± 0·60.

Effect of thiorphan

The IC50 value of leu-enkephalin was not altered by incubating the tissues with the enkephalinase inhibitor thiorphan (10 μM, 10 min, $P > 0.05$, n = 3).

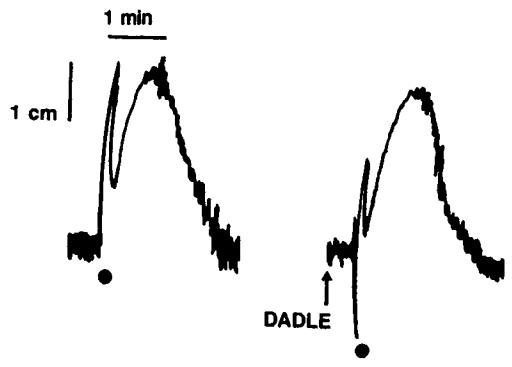

FIG. 1. Representative trace showing the effect of DADLE on the response to electrical stimulation of the enteric nerves of rat isolated jejunum. The control response on the left comprised a fast contraction which peaked during the 8 s stimulation at 10 Hz then partially recovered before contracting again. Following tissue recovery by 5 min, DADLE (10 nM, 1-min incubation) revealed a fast relaxation and inhibited the fast contraction during the period of stimulation. The slow secondary after-contraction was unaltered by DADLE.

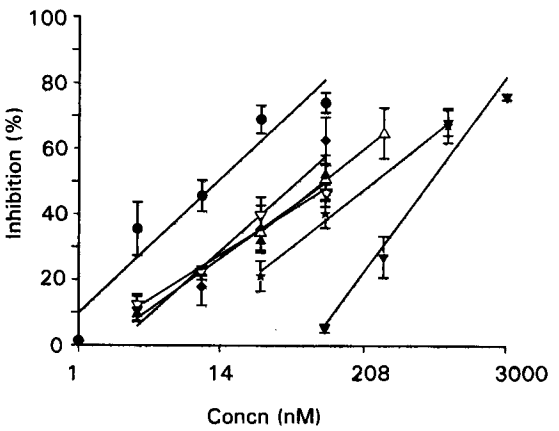

FIG. 2. The effect of the opioid peptides in inhibiting the cholinergic response of segments of rat jejunum. From most to least potent: DSLET (●), DADLE (◆), dynorphin A (△) metenkephalin (▲), leu-enkephalin (▽), DPDPE (★), DAGO (▼). Bars indicate the s.e. of the means. The IC50 values derived from these data are listed in Table 1.

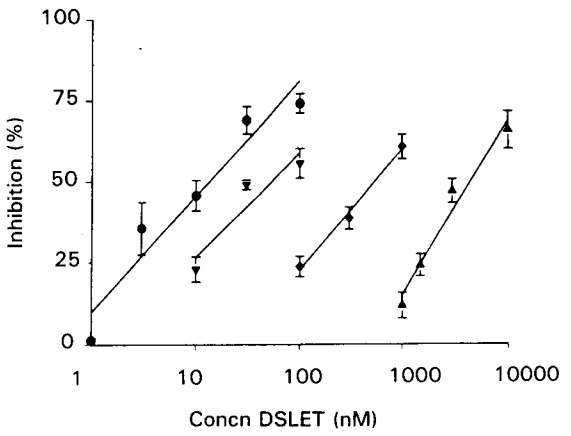

FIG. 3. Effect of naltrindole on the inhibitory effect of DSLET on the cholinergic response of the rat isolated jejunum to 10 Hz for 8 s. Full circles on the left show the control regression line for DSLET (n = 36). Naltrindole (15-min incubations: 0·2 nM ▼, n = 11; 2 nM ◆, n = 12; 20 nM ▲, n = 15) caused a concentration-related inhibition of DSLET. Bars indicate the s.e. of the means.

Discussion

This study provides evidence that the previously identified δ-opioid receptors in the rat intestine (Nishimura et al 1986) have a role in suppressing acetylcholine release from enteric cholinergic neurons. This was established using the criteria of agonist potencies and susceptibility to a selective antagonist.

The δ-sensitive response has been characterized as cholinergic, since this study has established that it is blocked by atropine and potentiated by neostigmine. Further, the δ-agonists act presynaptically to inhibit acetylcholine release since Coupar & De Luca (1994) found that DADLE up to 3 μM did not affect contractions produced by exogenous acetylcholine.

The order of potencies of the agonists in the rat intestine were found to be similar to their orders previously observed in the mouse vas deferens by Corbett et al (1984). This group used the mouse vas deferens (δ-rich)/guinea-pig ileum (μ- and κ-rich) bioassays to determine the selectivities of δ-opioid peptides. DADLE was shown to have moderate selectivity for δ-receptors (selectivity ratio = (IC50 mouse vas)/(IC50 guinea-pig ileum) = 0·08). The peptides DPDPE and DSLET were shown to have higher selectivity ratios (0·001 and 0·005, respectively) and high affinity ratios in binding assays (Rees & Hunter 1990). However, in spite of the high selectivity of DPDPE, it has been shown to have lower intrinsic activity compared with, say, DADLE (Rees & Hunter 1990), a property which is illustrated by the present results.

Further evidence showing the presence of functional δ-receptors in the rat small intestine has been gained by the use of the highly selective non-peptide δ-opiate antagonist naltrindole. In the present study naltrindole was shown to be a potent and competitive antagonist of the selective opioid agonist DSLET with a pK_B value of 10·22. This value is not significantly different from a previously published pK_B value of 9·74 for naltrindole at the δ-receptor, but is significantly different from previously reported pK_B values for naltrindole at κ-receptors (7·5) and μ-receptors (8·3) in the mouse vas deferens (Rogers et al 1990).

The effect of opioid peptides with activities at μ- and κ-receptors have also been investigated in the present study. DAGO, which is a relatively selective and potent μ-receptor agonist, was the least potent of the peptides in the rat intestine with an IC50 52 times that of DSLET. Similarly, DAGO has been shown to be considerably less potent than DSLET in the mouse vas deferens (Corbett et al 1984).

Dynorphin A is predominantly a κ-agonist in the guinea-pig ileum (Chavkin et al 1982) but also shows significant agonist activity at δ-receptors of the mouse vas deferens (IC50 0·33 nM; Corbett et al 1982). This would explain its inhibitory effect on the cholinergic contraction in the rat intestine found in this study. This appears to be the case, especially since there is no evidence in the literature to indicate the existence of κ-receptors in the rat intestine.

The potencies of the peptides in the rat intestine are low compared with their potencies in the mouse vas deferens; for instance, DSLET is 23 times more potent in the mouse vas deferens (Corbett et al 1984). However, the mouse vas deferens may be sensitive to δ-opioid peptides in comparison with other tissues. To illustrate this, the potencies of the peptides in the hamster vas deferens are similar to those found in this study for the rat intestine. For instance, DSLET is approximately 1·6 times more potent in the rat intestine than in the hamster vas deferens and this is in spite of the fact that peptidase enzymes were inhibited in the hamster study and not in the present one. In the absence of inhibitors, DSLET is considerably less potent in the hamster vas deferens than the rat small intestine (McKnight et al 1985).

The low potency of the peptides in the rat small intestine compared with the mouse vas deferens could be due to differences in endogenous peptidase activity. However, this is unlikely to be the explanation for the relatively low potencies of DADLE and DPDPE since they are not substrates for peptidases, at least not in the hamster vas deferens (McKnight et al 1985).

However, the potency values of the naturally-occurring peptides met- and leu-enkephalin in the rat small intestine are low and would be expected to be higher in the presence of peptidase inhibitors. A single group of experiments using the enkephalinase inhibitor thiorphan showed no significant effect on the response of leu-enkephalin in the rat intestine. However, in the hamster vas deferens a combination of peptidase inhibitors consisting of thiorphan, bestatin, captopril and L-leucyl-L-leucine increased the potency of leu-enkephalin at least 40-fold (McKnight et al 1985).

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