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The Peristaltic Reflex in the Rat Ileum: Evidence for Functional μ- and δ-Opiate Receptors

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Abstract

The potency order of opiate agonists at decreasing the rate of peristalsis in the rat isolated ileum was: difenoxin > loperamide > DADLE (D-Ala^2-D-Leu^5-enkephalin) > morphine > DSLET (D-Ser^2,Leu^5-Thr^6-enkephalin). U-50488 (*trans* 3,4-dichloro-*N*-methyl-*N*-(2-(l-pyrrolidinyl) cyclohexyl) benzeneacetamide methane sulphonate) was inactive at 300 nM. Naloxone (400 nM) caused a significant 1·52-fold increase in the rate of peristaltic contractions and inhibited the effects of the active opiate agonists. The apparent pA_2 values of naloxone were similar using difenoxin, loperamide and morphine as agonists, but the value was slightly, though significantly lower when DADLE was the agonist.

It is suggested that the previously identified δ-opiate receptors of the rat small intestine have a functional role in suppressing the peristaltic reflex. The same response is subserved by μ-opiate receptors and either of these opiate-receptor subtypes could be activated by endogenous enkephalins.

The ileum from the guinea-pig has been used extensively to study both physiological and pharmacological aspects of the peristaltic reflex (see Costa & Furness 1982). These numerous studies have established that μ- and κ-opiate receptors are located on the final cholinergic neurones of this tissue where they have a role in modulating acetylcholine release. This later conclusion is based on data derived from experiments which measured the effect of selective agonists and antagonists on the twitch response of the isolated ileum to low frequency transmural stimulation rather than functional peristaltic contractions (Paton 1957; Hutchinson et al 1975; Lord et al 1977; Collier et al 1981). It has been recognized for some time that the response of the ilea of other species to morphine differs markedly from that of the guinea-pig (Weinstock 1971). Despite this, there are only a few reports concerning the possible effects of opiates on isolated intestines of laboratory animals other than the guinea-pig. For instance, it has been shown that morphine does not inhibit low-frequency cholinergic transmission in the ileum of the mouse (Smith et al 1988) and the rabbit (Oka 1980) as it does in the guinea-pig (Paton 1957). Further, distinct differences have been noted in the effect of naloxone on the peristaltic reflex of the rat, cat and dog small intestines compared with the guinea-pig (Kromer et al 1979). Of these limited studies none have examined the neuromodulatory or opiate-receptor subtype in the different tissues.

Autoradiographic studies of the rat ileum have revealed that it contains a heterogeneous population of opiate receptors. Whereas μ-receptors are common to both the rat and guinea-pig ileum a striking difference exists in the second subtype which is the δ-receptor in the rat ileum as opposed to the κ-opiate receptor of the guinea-pig ileum (Monferini et al 1981; Dashwood et al 1985; Nishimura et al 1986).

Our studies have shown that the occupation of these opiate receptors by δ-selective enkephalin analogues leads to a functional reduction in transmurally-released acetylcholine from the enteric neurons, using the activity of the longitudinal muscle as an index of the response (Hancock & Coupar 1994). However, the cholinergic contractile response to transmural stimulation is not inhibited by μ-selective agonists (Coupar & DeLuca 1994).

The aim of the present study was to determine whether the δ-receptors are a functional part of the peristaltic reflex arc in the rat ileum. In addition, the possibility that functional μ-receptors may be located proximal to the final cholinergic neurons in the reflex arc is also addressed.

Materials and Methods

Tissue preparation

Segments of ileum (5 cm proximal to the caecum) were removed from 300–400 g rats and tied at the aboral end onto a glass J-tube. Each tissue was placed in a 25-mL jacketed organ-bath containing Krebs–Henseleit 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, maintained at 37°C and gassed with 5% CO_2 in O_2. Segments were tied off at the oral end to give lengths of between 6 and 8·5 cm and the cotton thread was connected to an isotonic transducer (Ugo Basil) to monitor length changes of the longitudinal smooth muscle under a resting load of 1 g. The free end of the J-tube was connected by flexible tubing to a 5-mL float chamber half filled with Krebs–Henseleit solution. The float was connected to a second isotonic transducer to monitor volume changes related to circular muscle contraction. Both the reservoir and transducer (volume meter) were attached to a worm-screw stand, enabling them to be raised above the fluid level of the organ bath as in the Trendelenberg (1917) method. Changes in longitudinal muscle contraction and volume

expulsion of the strips were displayed on a Grass model 79D polygraph.

Experimental protocol

After a 45-min equilibration period, the intraluminal pressure was increased gradually at approximately 3 mm s^{-1} until it initiated a peristaltic wave. This was recorded as a contraction of the longitudinal muscle (preparatory phase) closely followed by expulsion of fluid (circular muscle contraction). The pressure resulting in a peristaltic contraction was maintained for 15 min. Drugs were then added cumulatively to the bath fluid until an obvious change in the rate of peristaltic contractions occurred. The bath fluid was then changed and the segments were equilibrated at zero pressure for 10 min. The segments were reinflated for a further 15 min to measure the effects of another test drug.

Drugs

Atropine sulphate (Sigma, Castle Hill, Australia), D-Ala^2-D-Leu^5-enkephalin (DADLE, Bachem, Bubendorf, Switzerland), D-Ser^2,Leu^5,Thr^6-enkephalin (DSLET, Auspep, Melbourne, Australia), morphine hydrochloride (MacFarlane-Smith, Edinburgh, UK), naloxone hydrochloride (Du Pont Merck, Wilmington, USA), U-50488 (*trans* 3,4-dichloro-*N*-methyl-*N*-(2-[1-pyrrolidinyl] cyclohexyl) benzeneacetamide methane sulphonate, Upjohn, Kalamazoo, USA) were dissolved in distilled water. Loperamide HCl (Ethnor, Sydney, Australia) was dissolved in 10% ethanol in distilled water and difenoxin HCl (Janssen-Cilag, Sydney, Australia), was dissolved in ethanol. All drug solutions were added to the bath. The concentration of ethanol in the bath did not exceed 0·1%.

Statistics

Linear regression analysis was used to determine the potencies of the agonists which are expressed as IC50 values (with 95% confidence intervals) defined as the concentration causing a 50% reduction in the rate of peristalsis. IC50 values were also derived for the agonists in the presence of a single concentration of naloxone (400 nM for morphine and difenoxin; 40 nM for DADLE and loperamide). The resultant dose ratios (DR) were used to calculate the apparent pA_2 values from the K_B values derived from equation 1.

$$K_B = B/DR - 1 \quad (1)$$

where -log K_B is the apparent pA_2 and B is the concentration of naloxone. Student's paired *t*-test was used to assess the effect of naloxone on the rate of peristalsis.

Results

A mean pressure of 8 ± 0·2 cm H_2O (n = 4) was required to initiate rhythmic contractions of the longitudinal muscle and associated volume expulsions in the control preparations of the rat ileum. The frequency of these parameters remained the same over 15 min at 0·8 ± 0·1 peristaltic waves min^{-1} (n = 4) and each resulted in an 18 ± 2% (n = 4) shortening of the longitudinal muscle and expulsion of 104 ± 13 μL fluid per cm of tissue length (n = 4). Atropine (100 nM, n = 3) abolished the contractions and volume expulsions.

The opiate agonists, except U-50488, decreased the rate of peristalsis with the following order of potency: difenoxin < loperamide < DADLE < morphine < DSLET. U-50488 was inactive up to 300 nM. The IC50 values are shown in Table 1.

Naloxone (400 nM) caused a rapid 1·5 ± 0·1-fold increase in the rate of peristalsis ($P < 0·05$, Student's *t*-test, n = 6). Naloxone (10-min incubation) also inhibited the effects of the active opiate agonists. The apparent pA_2 values of naloxone were similar using difenoxin, loperamide and morphine as agonists, but were significantly lower when DADLE was used (Table 1).

Discussion

This study shows that the δ-opiate receptors of the rat small intestine have a functional role in suppressing the peristaltic reflex. In addition, μ-opiate receptors subserve the same response. Evidence is also provided that either or both receptor subtypes are activated by endogenous enkephalins.

The presence of functional δ-receptors is shown by the potent inhibitory effect of DADLE which has moderate selectivity for δ-receptors (selectivity ratio = IC50 mouse vas deferens/IC50 guinea-pig ileum = 0·08). Also DSLET which has a higher selectivity ratio (0·005; Corbett et al 1984) and a higher affinity ratio in binding assays (Rees & Hunter 1990) was also reasonably potent. The IC50 value of DADLE for inhibition of peristalsis corresponds to the value for inhibiting the cholinergic contraction of the rat jejunum in response to transmural stimulation (43 vs 38 nM; Hancock & Coupar 1994). This response to transmural

Table 1. Potencies of agonists at inhibiting the peristaltic reflex and affinity values of naloxone.

Agonist	IC50 (nM) (95% CI)	n	Naloxone pA_2 (95% CI)	n
Morphine	117 (115–118)	5	8·39 (8·32–8·45)	11
Difenoxin	10·4 (7·8–13·1)	5	8·61 (8·51–8·74)	10
Loperamide	16·6 (15–18·2)	4	8·47 (8·42–8·53)	8
DADLE	43·1 (41·3–44·9)	5	8·23 (8·20–8·26)	10
DSLET	116 (109–123)	7		
U-50488	>300			

IC50 values represent the concentration of agonist causing 50% inhibition in the rate of peristalsis (both longitudinal muscle contraction and volume expansion were used) calculated by regression analysis. The apparent pA_2 values of naloxone were calculated from the dose ratio produced by a single concentration of naloxone.

stimulation only involves activation of δ-receptors since μ- and κ-selective agonists are inactive in this preparation. Further, the order of agonist potencies corresponds to action at δ-receptors as does the affinity value of the δ-selective antagonist naltrindole using DADLE as agonist (Hancock & Coupar 1994). However, the IC50 of DSLET was higher in the peristaltic reflex than transmural stimulation (116 vs 13·5 nM; Hancock & Coupar 1994). This difference is due to the susceptibility of DSLET to peptidases and the need to incubate it for a longer time in experiments investigating the peristaltic reflex compared with experiments using transmural stimulation (Hancock, unpublished results). The finding that the pA_2 value of naloxone was significantly lower using DADLE as the agonist compared with the values using the predominantly μ-selective agonists morphine, difenoxin and loperamide could be taken as further evidence supporting the existence of functional δ-receptors. However, naloxone shows only a small (approx. 10-fold) discrimination in bioassays towards μ- compared with δ-receptors (Leslie 1987). In spite of this, the apparent pA_2 values for naloxone are an indication that the agonists interact with opiate receptors and that a blocking concentration reveals an inhibitory effect of endogenous opioids.

The presence of functional μ-receptors is confirmed by the results that morphine and the antidiarrhoeal drugs are potent inhibitors of the rat ileum peristaltic reflex. Morphine appears to be less potent at inhibiting peristalsis in the guinea-pig than in the rat (842 vs 117 nM (Van Neuten 1968; present results)), but it has the same potency at inhibiting twitch responses in transmurally stimulated guinea-pig ileum (112 nM (Rees & Hunter 1990)). The potency of difenoxin is in the same range for inhibition of rat ileum peristalsis as for inhibition of peristalsis in the guinea-pig ileum (IC50 = 2·9 nM (Van Nueten & Janssen 1972)) and the potency of loperamide is essentially the same in both tissues (guinea-pig ileum IC50 = 14 nM (Van Nueten et al 1974)).

The apparent pA_2 values of naloxone recorded in the rat ileum against the predominantly μ-selective agonists agree with previously reported values of 8·72 and 8·58 (slopes constrained to -1) at μ-receptors in the transmurally stimulated guinea-pig ileum (Lord et al 1977; Tallarida et al 1982). Additionally, the value obtained for naloxone in the present study using morphine as the agonist is statistically the same as the apparent pA_2 value derived in the guinea-pig ileum peristaltic reflex preparation (8·39 vs 8·5 (Tonini et al 1992)).

Our previous study using transmurally stimulated rat ileum failed to show activity of the κ-selective agonist ethylketocyclazacine (Coupar & DeLuca 1994). Similarly, the present study does not reveal functional κ-receptors since the highly selective κ-agonist U-50488 did not inhibit peristalsis at a concentration 75 times higher than its IC50 in the transmurally stimulated guinea-pig ileum (Rees & Hunter 1990).

Our previous finding that naloxone (100 nM) potentiated contractions of the rat ileum in response to transmural stimulation was cited as evidence that endogenous enkephalins could have a functional role in attenuating cholinergic nerve activity in this model (Coupar & DeLuca 1994). Enkephalin-containing nerves are present throughout the gut of many species (Kromer 1989). In the rat small intestine there is a relatively high density of fibres in the mucosa, submucosa and circular muscle (Schultzberg et al 1980; Ekbland et al 1991). Therefore, the present finding that naloxone increases the rate of peristaltic contractions supports the view that these enkephalinergic nerves are functionally involved in modulating peristalsis in the rat ileum.

This and previous studies have established that there are distinct differences between the ileum of the guinea-pig and rat as regards the receptors at which opiates act to suppress the peristaltic reflex. Although there is biochemical, electrophysiological and limited functional evidence for the presence of δ-receptors in the guinea-pig ileum, they are not considered to play a significant role in the control of peristalsis (Kromer 1990; Waterman et al 1992; see also Leslie 1987 for review). Stimulation of δ-receptors in the guinea-pig ileum does not affect the emptying phase of peristalsis but does cause a slight reduction in longitudinal muscle contraction (preparatory phase). It has been suggested that the site of this effect is either sensory neurons or interneurons (Waterman et al 1992). In the rat the δ-receptor stimulation abolishes peristaltic emptying and the receptors would appear to be located on the final cholinergic neurons of the reflex arc because δ-agonists inhibit the cholinergic contraction in response to transmural stimulation (Coupar & DeLuca 1994). Conversely, it can be assumed that μ-opiate receptors are located proximal to the final cholinergic neurons in the rat ileum, since μ-agonists are potent at inhibiting peristalsis but not the cholinergic contractile response to transmural stimulation (Coupar & DeLuca 1994).

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