Modulation of peristalsis in the guinea-pig isolated small intestine by exogenous and endogenous opioids

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1 A recording method was developed to measure physiological parameters of the preparatory and emptying phases of peristalsis *in vitro*. This method enabled measurement of: the compliance of the intestinal wall during the preparatory phase (a reflection of the resistance of the wall to distension); longitudinal muscle contraction during the preparatory phase; the threshold volume required to trigger the emptying phase; the maximal ejection pressure and the average power generated during the emptying phase, which reflects the rate at which the intestine performs work. Modulation of these parameters by exogenous and endogenous opioids acting at μ , κ and δ opioid receptors was investigated.

2 The compliance of the intestinal wall during the preparatory phase was reduced by the μ opioid receptor agonist, [D-Ala², N-methyl-Phe⁴, Gly⁵-ol] enkephalin (DAMGO) but not by the κ agonist, dynorphin, or the δ agonist, [D-penicillamine², D-penicillamine³] enkephalin (DPDPE). Reflex contraction of the longitudinal muscle during the preparatory phase was inhibited by DAMGO, dynorphin and DPDPE. The threshold volume required to trigger the emptying phase of peristalsis was increased by DAMGO, dynorphin and DPDPE.

3 The maximal ejection pressure generated during the emptying phase was reduced by dynorphin and DPDPE, but not by DAMGO. The average power generated by the intestine when emptying was not altered by any of the agonists.

4 Electrically stimulated contractions of longitudinal muscle in strips of longitudinal muscle-myenteric plexus were not inhibited by DPDPE. Similarly, DPDPE did not significantly inhibit electrically induced contraction of circular muscle in strips of circular muscle-myenteric plexus.

5 Each of the agonist effects on peristaltic parameters was antagonized by the appropriate antagonist: D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH₂ (CTOP) (μ), norbinaltorphimine (nor-BNI) (κ), naltrindole (δ).

6 It is concluded that μ and κ agonists act primarily on excitatory circular and longitudinal muscle motor neurones. The δ agonist probably acts on enteric neurones presynaptic to excitatory circular and longitudinal muscle motor neurones.

7 Antagonists for μ , δ and κ receptors did not affect any parameters of peristalsis when the intestine emptied against a low resistance. However, when emptying against a high outflow resistance, the average power generated by the intestine was increased by the κ antagonist, nor-BNI, but not by CTOP or naltrindole.

8 It is concluded that endogenous opioids appear to have little role in peristalsis when the intestine is working against a low outflow resistance. However endogenous opioids, acting primarily at κ receptors, provide a braking mechanism by inhibiting the emptying phase of peristalsis in conditions in which the intestine empties against a higher resistance.

Keywords: Intestinal motility; endogenous opiates; opioid peptides

Introduction

Peristalsis is an important pattern of motility occurring in the intestine (Cannon, 1912; Kosterlitz & Lees, 1964), involving the coordinated contraction of smooth muscle in both the circular and longitudinal muscle layers to propel intestinal contents in an aboral direction. This activity occurs in isolated segments of intestine and is controlled by the enteric nervous system (Tonini *et al.*, 1981; Costa & Furness, 1982). When fluid is infused continuously into the lumen, the intestine gradually distends (the preparatory phase of peristalsis) until, at a threshold distension, the circular muscle at the oral end of the intestine contracts and a wave of contraction propagates aborally along the intestine (the emptying phase; Kosterlitz & Lees, 1964). Once the intestine has emptied, the cycle starts again if the infusion of fluid continues.

Exogenous opioids alter intestinal motility both *in vivo* and *in vitro* (Kromer, 1988; Miller & Hirning, 1989). Furthermore, endogenous opioids exist in the enteric nervous system of many mammalian species (Hughes *et al.*, 1977; Linnoila *et al.*, 1978; Furness *et al.*, 1983; Costa *et al.*, 1985; Corbett *et al.*, 1988). Opioid receptors have been shown to be present by ligand binding studies (Leslie *et al.*, 1980; Goldstein & Naidu, 1989), electrophysiology (North & Tonini, 1977; Galligan & North, 1991; Pillai & Johnson, 1991) and pharmacology (e.g. Greenberg *et al.*, 1970; Kosterlitz & Waterfield, 1972; Dingleline & Goldstein, 1976). In the guinea-pig ileum, activation of the μ and κ subtypes of opioid receptor on neurones inhibits nerve-mediated contraction of both circular muscle and longitudinal muscle (Kosterlitz & Waterfield, 1972; Johnson *et al.*, 1987) and inhibits peristalsis *in vitro* (Trendelenburg, 1917; Holzer & Lembeck, 1979; Kromer *et al.*, 1980). Specific effects of agonists at the δ receptor subtype on motility in the guinea-pig ileum have been reported in few studies (Kromer, 1988; 1990).

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Endogenous opioids are released from enteric neurones during peristalsis (Donnerer *et al.*, 1984) and in response to electrical stimulation (Puig *et al.*, 1977; Glass *et al.*, 1986). The role of endogenous opioids in peristalsis has previously been studied by use of the Trendelenburg preparation or modifications thereof (Van Nueten *et al.*, 1976; Kromer, 1990). However, these methods do not match normal physiological activity, i.e. only the aboral end is open, and fluid inflow and outflow occur through this opening. Hence in this arrangement, the intestine is effectively a blind sac and intestinal contractions force fluid against the inflow head of pressure. At low distending pressures, peristaltic activity rapidly ceases; at higher distending pressures, intermittent, tetrodotoxin-resistant contractile activity is observed (Davison & Najafi-Farashah, 1982). This inhibition of peristalsis has been described as 'fatigue' and has been attributed to the release of endogenous opioids (Van Nueten *et al.*, 1973). However, these experiments were not able to address the role of endogenous opioid peptides in more physiological conditions. Tonini *et al.* (1981) modified the method of Büllbring *et al.* (1958) to study peristalsis. In this modification, the intestine is open-ended; fluid is pumped into the oral end of the intestine and expelled through the aboral end by peristaltic contractions. A one-way valve at the aboral end ensures that expelled fluid does not flow back into the intestine. The method allows a more physiological peristalsis to be studied continuously for several hours without the rapid cessation of contraction seen in previous studies. Based on this technique, we have developed a recording method for measuring physiological parameters of peristalsis *in vitro* (Waterman *et al.*, 1990) and have used this to study the site and mode of action of opioid agonists at μ , κ and δ receptor subtypes and to investigate the possibility that endogenous opioids may have a role in peristalsis.

Methods

Guinea-pigs (strain: IMVS coloured) of either sex and weighing between 250 and 350 g were stunned and bled via the carotid arteries. Segments of ileum just distal to the ligament of Treitz were removed, flushed of luminal contents and placed in Krebs solution at room temperature, gassed with 95% O₂/5% CO₂, until required. The composition of the Krebs solution was (mM): NaCl 118, KCl 4.7, NaH₂PO₄ 1.0, NaHCO₃ 25, MgSO₄ 1.2, D-glucose 5.5 and CaCl₂ 2.5, pH 7.4.

Peristalsis

Peristalsis was studied by the method of Tonini *et al.* (1981). Segments of ileum, approximately 7–8 cm in length, were mounted horizontally in a 100 ml organ bath. The organ bath contained Krebs solution maintained at 36–37°C and gassed with 95% O₂/5% CO₂. The Krebs solution was replaced at least every 30 min. Peristalsis was elicited by pumping Krebs solution into the oral end of the intestinal lumen.

Longitudinal muscle contraction was recorded with a Harvard Bioscience isotonic transducer with tension set at 7.5 mN. Intraluminal pressures at the oral and aboral ends of the length of intestine were measured with three Statham pressure transducers. Two transducers measured aboral pressure; one recorded at high sensitivity and the other at low sensitivity. Traces were displayed on a Grass polygraph (Figure 1). The volume expelled at the end of each wave of contraction was measured with a measuring cylinder. The residual volume remaining in the intestine after a peristaltic wave could be estimated by siphoning fluid from the intestine with a fixed length of tubing at the end of each experiment. The intraluminal pressure following removal of the residual volume was defined as the baseline pressure and the length of intestine under these conditions was considered to be the resting length.

Figure 1 Polygraph recording of peristalsis in the guinea-pig isolated small intestine.

The flow rates used were either 0.63 ml min⁻¹ (low rate) or 3.43 ml min⁻¹ (high rate). The high flow rate was equivalent to approximately half the average flow rate generated by the intestine itself when emptying. Each peristaltic wave caused the expulsion of fluid from the aboral end of the intestine via a one-way valve. Distal to the valve a length of rigid tubing acted as a resistance to fluid outflow; changing the length of the tubing altered the total outflow resistance. The increase in resistance resulting from lengthening the outflow tubing was estimated in the following manner. A piece of rigid tubing was mounted in the organ bath instead of intestine and the intraluminal pressure measured when different lengths of outflow tubing were in place. Since pressure and resistance are directly proportional if flow rate is constant, an increase in pressure of 21% in response to increasing the length of the tubing indicates that there is a 21% increase in resistance. Fluid leaving the end of the tubing activated a balanced switch that switched off the infusion pump for 10 s to allow the intestine time to finish emptying before more fluid entered.

Physiological parameters of peristalsis

Two phases of peristalsis can be identified: the preparatory phase, when the intestine is being filled with fluid, causing the longitudinal muscle to contract, and the emptying phase, when the circular muscle contracts and forces fluid from the aboral end (see Kosterlitz & Lees, 1964). A number of parameters relating to both phases were calculated from the records (Figure 2).

The compliance of the intestinal wall during the preparatory phase is defined as the change in intraluminal pressure in response to a given change in intraluminal volume (Guyton, 1986). Compliance is a reflection of the resistance of the intestinal wall to infused fluid and can be calculated from the inverse of the slope of the preparatory phase of the intraluminal pressure trace.

$$\begin{aligned} \text{average compliance} &= \frac{\text{volume infused in preparatory phase}}{\text{change in pressure in preparatory phase}} \\ &= \frac{\text{duration of preparatory phase} \times \text{inflow rate}}{\Delta P} \end{aligned}$$

The emptying phase of peristalsis is elicited when the intestine is radially distended to a threshold level. There does not appear to be a pressure threshold (Kosterlitz & Robinson, 1959; Ginzl, 1959). The threshold was therefore measured as the volume of fluid in the intestine when the emptying phase was initiated. The threshold volume consists of the volume infused into the intestine during the preparatory phase

(= duration of preparatory phase $\times$ inflow rate) plus the residual volume. In control tissue, the residual volume represents fluid trapped between folds of mucosa and was calculated by siphoning out the contents of the intestine after a peristaltic wave. Changes in residual volume were estimated to be the difference between the volume infused and the volume expelled from the intestine during a peristaltic wave. No significant changes in residual volume were observed in the absence of drugs.

Two parameters of the emptying phase were measured: the maximal ejection pressure (Figure 2) and the average power generated by the intestine when emptying. A positive linear correlation was found between the maximal ejection pressure and the length of segment of intestine. This may reflect the larger volume expelled from the longer segments. To account for slight differences in length of each segment, maximal ejection pressure is therefore expressed per length of intestine. Average power can be defined as pressure $\times$ flow rate (Nave & Nave, 1980) and is calculated as:

$$\text{power} = \frac{\text{average pressure in emptying phase} \times \text{volume expelled}}{\text{duration of emptying phase}}$$

The average power generated by the intestine was linearly correlated with the length of intestine. This probably occurs because longer segments of intestine expel a greater volume and because the duration of the emptying phase is longer when a peristaltic wave has further to travel. Consequently, average power is also expressed per length of intestine.

Longitudinal muscle contraction was calculated as the percentage shortening of the intestine at threshold, relative to the resting length.

Circular muscle and longitudinal muscle contraction

The circular muscle-myenteric plexus (CM-MP) preparation of Johnson *et al.* (1987) and a modified version of the longitudinal muscle-myenteric plexus (LM-MP) preparation of Rang (1964) were used to investigate further the actions of the opioid receptor agonists on circular and longitudinal muscle contraction. In the CM-MP preparation, strips of distal ileum, 10 mm in length, from which the mucosa and submucosa had been removed, were mounted in a 5 ml organ bath containing Krebs solution bubbled with 95% O₂/5% CO₂. Circular muscle contractions were recorded with a Harvard isotonic transducer with tension set at 7.5 mN. Following a 90 min equilibration period, the preparations were

Figure 2 Schematic peristaltic wave showing variables measured in order to calculate values of physiological parameters of peristalsis as described in text.

stimulated electrically via a pair of platinum ring electrodes by a Grass S44 stimulator. The stimulus frequency was 1 pulse every 2 min, pulse duration 0.3 ms and supramaximal pulse amplitude (60 V).

In the LM-MP preparation, a 2 cm strip of longitudinal muscle-myenteric plexus was folded in half and mounted in a 5 ml organ bath containing Krebs solution. Isotonic contractions of the longitudinal muscle were recorded with a Harvard isotonic transducer with 750 mg counterweight. After a 45 min equilibration period, the strips were stimulated via a pair of platinum ring electrodes with a Grass S44 stimulator. Square wave pulses of supramaximal amplitude (60 V) and 0.5 ms duration were delivered at a frequency of 0.1 Hz. Some opioid receptor agonists probably inhibit neuromuscular transmission by hyperpolarizing neuronal membranes (North & Egan, 1983). Therefore the use of a supramaximal stimulus amplitude may overcome the hyperpolarization and bring the membrane to threshold for initiation of an action potential. In some experiments, the amplitude of the pulses was therefore reduced to produce 50% of the maximal contraction (i.e. EV₅₀).

Experimental design and analysis of results

Data are presented as the means $\pm$ s.e. means of experiments using *n* guinea-pigs. Each measurement was made with a separate segment of intestine. Control activity was recorded for at least 5 min before the addition of an agonist. After a further 5 min, an antagonist was added. In some experiments, the antagonist was added 3 min (D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH₂ (CTOP)) or 12 min (nor-binaltorphimine (nor-BNI) and naltrindole) before the agonist. The maximal effect of CTOP occurs within 5 min (Shook *et al.*, 1987). In experiments comparing the effect of an opioid agonist with control recordings, data were analysed with a paired *t* test (two-tailed). Multivariate analysis of variance for repeated measures was used to analyse results from experiments involving control, antagonist and agonist measurements. The General Linear Model procedure of the SAS/STAT System for personal computers (SAS Institute Inc, Cary, NC, 1988) was used. A probability of less than 0.05 was regarded as significant.

Drugs

The following drugs were used: hexamethonium bromide, [D-Ala², N-methyl-Phe⁴, Gly⁵-ol]enkephalin (DAMGO) and tetrodotoxin (Sigma, St Louis, U.S.A.); dynorphin A-(1-13) (Auspep, Melbourne, Australia); [D-Penicillamine², D-Penicillamine⁵]enkephalin (DPDPE) and D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH₂ (CTOP) (Peninsula, Belmont, Cal, U.S.A.); nor-binaltorphimine (nor-BNI) and naltrindole (RBI, Mass., U.S.A.); and naloxone (Endo Laboratories, New York, U.S.A.).

The peptides were dissolved in 12% trifluoroacetic acid and 0.1% dithiothreitol. At the final concentrations in the bath, these solvents had no effect on the intestinal responses under investigation. The remaining drugs were dissolved in distilled water. The solutions were stored at -4°C and used within 2 weeks (nor-BNI) or 6 weeks (all other drugs). All drugs were administered in volumes less than 1% of the organ bath volume.

Results

Effects of the μ -opioid receptor agonist, DAMGO

The emptying phase of peristalsis was abolished by 200 nM DAMGO in 9 out of 15 preparations. Following addition of the drug, the intestine gradually filled with fluid until the intraluminal pressure increased sufficiently to overcome the outflow resistance. The fluid then moved straight through the

extremely distended intestine as if it were a passive tube. Longitudinal muscle contraction was completely inhibited. In 4 experiments, abolition of peristalsis revealed phasic activity of the circular muscle that was unaffected by tetrodotoxin and therefore probably myogenic. Peristaltic activity could be restored by the addition of $1\text{ }\mu\text{M}$ naloxone, but not by the κ -opioid receptor antagonist, nor-BNI (10 nM) or the δ -opioid receptor antagonist, naltrindole (200 nM). Values of the physiological parameters after peristalsis had been restored by naloxone were not significantly different to control values, with one exception. Contraction of the longitudinal muscle increased significantly from $12.1 \pm 1.5\%$ to $14.6 \pm 1.5\%$ ($n = 4$; $P < 0.05$). This represents acute dependence to exogenous opioids, as has previously been described by Chahl (1986).

In 6 out of 15 preparations, 200 nM DAMGO did not abolish the emptying phase of peristalsis. In these experiments, values for the physiological parameters of peristalsis were calculated before and after the addition of DAMGO. The compliance of the intestine during the preparatory phase was reduced from $6.9 \pm 1.0\text{ }\mu\text{l Pa}^{-1}$ to $4.2 \pm 0.7\text{ }\mu\text{l Pa}^{-1}$ ($n = 6$, $P < 0.05$). The threshold volume was increased by $6.9 \pm 0.6\text{ ml m}^{-1}$ ($n = 4$; $P < 0.001$). Immediately after the addition of DAMGO, the ensuing preparatory phase was prolonged until the new raised threshold volume was reached. The intestine then emptied to a new, increased, residual volume. The volume of fluid infused during subsequent preparatory phases was not significantly altered. In the experiments in which DAMGO did not abolish the emptying phase, the maximal ejection pressure and the average power generated by the intestine were not altered. Longitudinal muscle contraction was reduced from $14.1 \pm 2.5\%$ to $8.1 \pm 2.0\%$ ($n = 6$; $P < 0.05$).

Effects of the κ -opioid receptor agonist, dynorphin

Dynorphin (10 nM) abolished the emptying phase of peristalsis in 5 out of 12 experiments. Phasic contractions of the circular muscle were observed in one of these experiments. Peristalsis was restored by 10 nM nor-BNI or $1\text{ }\mu\text{M}$ naloxone, but not by 200 nM naltrindole. Each physiological parameter returned to control levels with the exception of longitudinal muscle contraction. The latter increased significantly from $9.8 \pm 1.3\%$ to $13.4 \pm 1.1\%$ ($n = 5$; $P < 0.02$) after exposure to dynorphin for approximately 5 min and indicates acute dependence to exogenous opioids, as described previously by Chahl (1986).

Physiological parameters of peristalsis before and after the addition of dynorphin were compared in the experiments in which the emptying phase of peristalsis was not abolished. Dynorphin did not significantly alter the compliance of the intestinal wall in the preparatory phase or the average power generated during the emptying phase. However, there was a large increase in the residual volume. Thus the threshold volume necessary to elicit the emptying phase increased by $5.6 \pm 1.4\text{ ml m}^{-1}$ ($n = 6$; $P < 0.02$). There was an increase in the volume of fluid infused into the intestine during the first preparatory phase after the addition of dynorphin, but not in subsequent preparatory phases. Dynorphin reduced the maximal ejection pressure from $30.0 \pm 1.7\text{ kPa m}^{-1}$ to $21.7 \pm 3.8\text{ kPa m}^{-1}$ ($n = 6$; $P < 0.01$). Longitudinal muscle contraction amplitude at the point of threshold was reduced from $12.0 \pm 2.5\%$ to $4.1 \pm 1.8\%$ ($n = 6$; $P < 0.01$).

The inhibitory effect of dynorphin on maximal ejection pressure could be due to an action of the drug on excitatory circular muscle motor neurones. In order to investigate this possibility, the effects of dynorphin were tested in the CM-MP preparation. Dynorphin (1 nM , which is the approximate IC_{50} ; Johnson, 1991) was added to the preparation following the addition of $30\text{ }\mu\text{M}$ hexamethonium to reduce ganglionic transmission. This concentration of hexamethonium is effective in blocking peristalsis (unpublished observations). Dynorphin significantly reduced the hexamethonium-resistant

contractions from $4.86 \pm 0.49\text{ mm}$ to $3.18 \pm 0.75\text{ mm}$ ($n = 6$; $P < 0.01$).

Effects of the δ -opioid receptor agonist, DPDPE

Unlike the μ - and κ -agonists, DPDPE did not abolish the emptying phase of peristalsis at concentrations up to $1\text{ }\mu\text{M}$. It did however significantly alter 3 of the 5 physiological parameters (Figure 3). Longitudinal muscle contraction was reduced from $9.4 \pm 0.9\%$ to $5.0 \pm 0.7\%$ ($n = 23$; $P < 0.0001$) by concentrations above 30 nM . Concentrations of DPDPE up to 77 nM did not affect threshold distension or maximal ejection pressure. Concentrations greater than 115 nM increased the threshold distension through an increase in the residual volume of $2.7 \pm 0.6\text{ ml m}^{-1}$ ($n = 15$; $P < 0.0001$). Maximal ejection pressure was reduced from $29.2 \pm 1.1\text{ kPa m}^{-1}$ to $25.8 \pm 1.1\text{ kPa m}^{-1}$ ($n = 12$; $P < 0.01$) by concentrations of DPDPE above 115 nM . Each of these effects was completely antagonized by the specific δ -opioid receptor antagonist, naltrindole, but not by the μ -(CTOP) or κ -(nor-BNI) receptor antagonists (Table 1). These effects are likely to reflect a very steep concentration-response curve. There was no significant effect of DPDPE on either the compliance of the intestinal wall or on the average power generated during the emptying phase.

Some of the effects of DPDPE could be due to an action of the drug on excitatory circular muscle motor neurones. To test this hypothesis, the effect of DPDPE on electrically stimulated contractions of circular muscle in strips of CM-MP was investigated. Circular muscle contraction was not significantly inhibited by concentrations of DPDPE up to $1\text{ }\mu\text{M}$.

Longitudinal muscle contraction was reduced by almost 50% by DPDPE during peristalsis. To investigate whether this was due to an action on excitatory longitudinal muscle motor neurones, the effect of DPDPE on electrically stimulated strips of LM-MP was investigated. There was no significant effect of DPDPE at any of the concentrations tested (up to $1\text{ }\mu\text{M}$). When the LM-MP strip was stimulated by a submaximal voltage (the EV_{50}), there was still no significant effect of DPDPE.

Effects of opioid receptor antagonists

The opioid receptor antagonists CTOP (μ), nor-BNI (κ) and naltrindole (δ), were used to reveal effects of endogenous opioids under different conditions of peristalsis. When fluid was infused into the intestine at a slow rate (0.63 ml min^{-1})

Figure 3 Effect of the δ -agonist, [D-penicillamine², D-penicillamine³]enkephalin (DPDPE) on peristalsis. The duration of the preparatory phase is increased in the first cycle after addition of DPDPE, indicating that a larger volume was infused to trigger the emptying phase. Maximal ejection pressure and shortening of the intestine were both reduced in all cycles following addition of DPDPE.

Table 1 Specificity of effect of [D-penicillamine², D-penicillamine⁵]enkephalin (DPDPE) on physiological parameters of peristalsis

Condition/Parameter	Change in residual volume (ml)	Maximal ejection pressure (kPa m ⁻¹)	Longitudinal muscle contraction (%)
Control	-0.013 ± 0.01 (15)	29.22 ± 1.13 (12)	9.39 ± 0.94 (23)
DPDPE	0.194 ± 0.03 (15)****	25.83 ± 1.11 (12)***	5.05 ± 0.68 (23)****
Control	0.012 ± 0.019 (18)	28.18 ± 0.85 (18)	9.49 ± 0.82 (18)
Naltrindole + DPDPE	0.062 ± 0.014 (18)*	27.96 ± 0.82 (18)*	9.18 ± 0.73 (18)*
Control	-0.025 ± 0.023 (4)	22.4 ± 3.29 (5)	9.49 ± 1.71 (5)
CTOP + DPDPE	0.085 ± 0.04 (4)**	18.09 ± 2.94 (5)**	4.38 ± 1.41 (5)****
Control	0.005 ± 0.06 (14)	29.62 ± 0.99 (14)	8.36 ± 5.91 (14)
Nor-BNI + DPDPE	0.21 ± 0.05 (14)**	27.16 ± 1.3 (14)**	5.91 ± 1.03 (14)****

The mean and the s.e.mean are given for experiments on (n) different animals. * $P > 0.05$; ** $P < 0.05$; *** $P < 0.01$; **** $P < 0.0001$. Probabilities refer to comparisons between DPDPE ± antagonist and the corresponding control. CTOP = D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH₂; nor-BNI = nor-binaltorphimine.

and the intestine emptied against a low resistance, there was no significant effect of any of the antagonists on any of the five parameters measured.

When the intestine was infused with fluid at the higher rate (3.43 ml min⁻¹), it emptied approximately three times more often than at the lower rate, but expelled the same volume of fluid and generated the same average power with each peristaltic wave. Under these conditions, there was also no significant effect of any of the antagonists on any of the parameters measured.

At the low inflow rate, the intestine generated significantly more average power when the resistance of the outflow tubing was increased by 21% by increasing the length of outflow tubing. In these conditions, nor-BNI increased the average power generated from 1.1 ± 0.1 mW m⁻¹ to 1.3 ± 0.1 mW m⁻¹ ($n = 14$; $P < 0.002$). There was no significant effect of nor-BNI on the other four parameters measured, nor were there significant effects of either of the other antagonists on other parameters of peristalsis. The effects of increasing both inflow rate and outflow resistance were not tested.

Discussion

Previous work has shown that in the guinea-pig ileum, opioids inhibit motility by acting on enteric neurones (North & Egan, 1983; Kromer, 1988). Peristalsis involves the co-ordinated activity of many functional classes of neurones on which opioids might act. The physiological parameters of peristalsis measured in this study reflect the activity of these classes of neurones. We have revealed multiple actions of exogenous and endogenous opioids in peristalsis. In order to interpret the actions of opioids, a brief discussion on the significance of the physiological parameters follows.

Physiological parameters of peristalsis

Radial distension of the intestine as it fills with fluid is detected by mechanoreceptors in the muscularis externa (Yokoyama & North, 1983; Smith *et al.*, 1990) and this must provide the underlying drive for peristalsis. During the preparatory phase, activity of circular muscle motor neurones determines the tone of the circular muscle and the resistance of the muscle to further distension. This was measured as the compliance of the intestinal wall. Activity of longitudinal muscle motor neurones during the preparatory phase causes longitudinal muscle contraction which was measured as shortening of the intestine. Once the threshold distension is reached, contraction of the circular muscle occurs and the threshold distension required to activate this reflex was measured in the present study. The extent of circular muscle contraction and its coordinated propagation determines the peak pressure and average power generated by the intestine.

The effect of selective opioid agonists will be considered on each of the measured parameters in turn, followed by effects of selective antagonists used to reveal the role of released endogenous opioids.

Compliance

The resistance of the intestinal wall to distension (subthreshold for initiation of the emptying phase of peristalsis) is determined by the passive resistance of the wall and the level of contractility of the muscle layer. Neither κ - nor δ -opioid agonists caused any measurable change in the compliance of the intestinal wall during the preparatory phase; however the μ -selective agonist DAMGO significantly reduced compliance. That is, following administration of DAMGO, any infused volume generated a greater luminal pressure change. μ agonists do not appear to act directly on the muscle in guinea-pig small intestine (Johnson *et al.*, 1987) and have been demonstrated to inhibit enteric neurones (North & Egan, 1983). Therefore these results suggest that the enteric inhibitory neurones are active during the preparatory phase and that the μ -agonist caused a decrease in the output of these neurones to the circular muscle. However, the site of action of DAMGO on compliance remains uncertain; it could inhibit motor neurones directly or reduce the excitability of sensory neurones or interneurones in the reflex pathway.

Longitudinal muscle contraction

Contraction of the longitudinal muscle layer during the preparatory phase of peristalsis was inhibited by μ -, κ - and δ -opioid agonists. This inhibitory effect of μ - and κ -agonists is likely to be due to inhibition of excitatory motor neurones to the longitudinal muscle layer (Greenberg *et al.*, 1970; Kosterlitz & Waterfield, 1972; Dingledine & Goldstein, 1976). However δ -agonists do not inhibit excitatory transmission to the longitudinal muscle in a comparable manner (Mosberg *et al.*, 1983; Galligan *et al.*, 1984; Shook *et al.*, 1987; Erspamer *et al.*, 1989; present study). This suggests that δ -agonists inhibit the reflex contraction of longitudinal muscle by acting on either sensory neurones or interneurones rather than directly on motor neurones.

Threshold for triggering the emptying phase

μ -, κ - and δ -opioid agonists all increased the threshold volume for triggering the emptying phase of peristalsis, in some cases abolishing the emptying phase altogether. In preparations where emptying was not abolished, the threshold volume for eliciting the emptying phase was significantly increased. Both μ - and κ -opioid agonists inhibit excitatory transmission to the circular muscle layer from circular muscle

motor neurones (Johnson *et al.*, 1987; 1988). Greater distension stimuli are known to result in a larger reflex contraction, probably due to a greater activation of the sensory, inter- and motor neurones in the ascending excitatory reflex of the guinea-pig small intestine (Tonini & Costa, 1990). This increase in activation of the reflex pathway may counteract the inhibitory effects of opioids. Similarly, in the preparation used in these experiments to study peristalsis, a greater distension stimulus would counteract the inhibitory effects of opioids on the excitatory motor neurones and trigger the emptying phase. As with the longitudinal muscle layer, δ -agonists do not inhibit excitatory motor neurone transmission to the circular muscle when the motor neurones are electrically stimulated (Johnson *et al.*, 1987; present study). Therefore it is likely that δ -agonists act on sensory neurones or interneurones involved in triggering the emptying phase rather than the final motor neurones.

Maximal ejection pressure and power generated during the emptying phase

During the emptying phase, the peak pressure generated was reduced by κ - and δ -agonists but not by the μ -agonist. These effects can be explained by an action of the κ -agonist on excitatory motor neurones to the circular muscle and of the δ -agonist on sensory neurones or interneurones, as for threshold volume. The lack of effect of the μ -agonist on maximal ejection pressure in contrast to the inhibition evoked by the κ -agonist suggests that there must be differences in the distribution of, or effects mediated by, μ - and κ -receptors on elements of the enteric neurones active during the emptying phase. However, the average power generated by the intestine was unaffected by all the three types of agonist, indicating that the intestine is able to empty equally effectively even after the maximal ejection pressure has been reduced. Nevertheless, there was a reduction in the proportion of the intraluminal volume that was emptied.

Specific effects of δ -opioid receptor agonists

The present study demonstrated several specific effects of DPDPE on motility, as was suggested for the less selective δ -agonist, [D-Ser²,Leu⁵]enkephalin-Thr (DSLET) (Kromer, 1990). The lowest effective concentration of DPDPE differed for each parameter, suggesting that DPDPE acts at more than one site in the reflex pathways underlying peristalsis, and/or that it acts on a single neuronal type which is common to several different pathways. The action of DPDPE could be deduced to be on sensory neurones or interneurones in the pathways innervating both the circular and longitudinal muscle layers, suggesting that these pathways may contain some neurones in common. Thus, using preparations to study peristalsis, δ -agonists have been demonstrated to have effects that were not apparent from studies in simpler preparations (Kromer, 1990; present study) and this reflects the variety of classes of neurones involved in peristalsis. This

method of studying peristalsis is highly suitable for revealing multiple sites of action of drugs on enteric neurones.

Modulation of peristalsis by endogenous opioids

Endogenous opioids are released from enteric neurones during peristalsis (Donnerer *et al.*, 1984). Using the methods in which the intestine is forced to empty against the inflow head of pressure (see Introduction), a depression in the amplitude of peristaltic contractions develops which is reversed by naloxone and hence was proposed to be mediated in part by endogenous opioids (Van Nueten *et al.*, 1976). These actions are likely to be mediated predominantly by κ -receptors (Kromer, 1990). Cessation of peristalsis under these conditions represents an extreme situation and has been described as fatigue. In the present study, a role for endogenous opioids was investigated in less extreme conditions. When the intestine was distended by a slow inflow of fluid at the oral end and the emptying at the aboral end was against a low resistance, there was no evidence for the release of endogenous opioids. Similarly, endogenous opioids did not have an effect when the intestine emptied three times as frequently, as a result of an increased rate of distension with fluid. However, when the intestine was forced to empty against a higher outflow resistance, thus being made to work harder, evidence was obtained for endogenous opioids reducing the average power generated by the intestine. This action was predominantly mediated by κ -receptors, and is consistent with the results obtained by Kromer (1990). We propose that endogenous opioids may function as a braking mechanism to prevent the intestine from generating excessive power in response to high resistances. The identification of the class of enteric neurones that is the source of these endogenous opioids remains to be established. However, both pro-enkephalin- and pro-dynorphin-derived peptides are found in different enteric neurones in this tissue (Hughes *et al.*, 1977; Linnoila *et al.*, 1978; Furness *et al.*, 1983; Costa *et al.*, 1985; Corbett *et al.*, 1988).

In conclusion, this study has demonstrated that endogenous opioids are released during peristalsis and reduce the average power generated by the intestine through an action at κ -receptors. This modulatory effect occurs only when the intestine is forced to empty against a high resistance. The study demonstrated specific effects of μ - and κ -opioid receptor agonists on physiological parameters of peristalsis which could be attributed to an action of these agonists on excitatory circular and longitudinal muscle motor neurones and specific effect of a δ -agonist on sensory neurones or interneurones which activate circular or longitudinal muscle motor neurones.

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