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Involvement of μ- and κ-, but not δ-, opioid receptors in the peristaltic motor depression caused by endogenous and exogenous opioids in the guinea-pig intestine

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1 Opiates inhibit gastrointestinal propulsion, but it is not clear which opioid receptor types are involved in this action. For this reason, the effect of opioid receptor–selective agonists and antagonists on intestinal peristalsis was studied.

2 Peristalsis in isolated segments of the guinea-pig small intestine was triggered by a rise of the intraluminal pressure and recorded *via* the intraluminal pressure changes associated with the peristaltic waves.

3 μ-Opioid receptor agonists (DAMGO, morphine), κ-opioid receptor agonists (ICI-204,448 and BRL-52,537) and a δ-opioid receptor agonist (SNC-80) inhibited peristalsis in a concentration-related manner as deduced from a rise of the peristaltic pressure threshold (PPT) and a diminution of peristaltic effectiveness.

4 Experiments with the δ-opioid receptor antagonists naltrindole (30 nM) and HS-378 (1 μM), the κ-opioid receptor antagonist nor-binaltorphimine (30 nM) and the μ-opioid receptor antagonist cyprodime (10 μM) revealed that the antiperistaltic effect of ICI-204,448 and BRL-52,537 was mediated by κ-opioid receptors and that of morphine and DAMGO by μ-opioid receptors. In contrast, the peristaltic motor inhibition caused by SNC-80 was unrelated to δ-opioid receptor activation.

5 Cyprodime and nor-binaltorphimine, but not naltrindole and HS-378, were *per se* able to stimulate intestinal peristalsis as deduced from a decrease in PPT.

6 The results show that the neural circuits controlling peristalsis in the guinea-pig small intestine are inhibited by endogenous and exogenous opioids acting *via* μ- and κ-, but not δ-, opioid receptors.

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Keywords: Intestinal peristalsis; δ-opioid receptors; κ-opioid receptors; μ-opioid receptors; opioid receptor agonists; opioid receptor antagonists; naltrindole; nor-binaltorphimine; cyprodime; enteric nervous system; constipation

Abbreviations: ANOVA, one-way analysis of variance; DAMGO; Tyr-D-Ala-Gly-NMe-Phe-Gly-ol; PPT, peristaltic pressure threshold

Introduction

Opiates have long been known to inhibit intestinal peristalsis (Trendelenburg, 1917) and to cause constipation. This effect is consistent with the presence of opioid peptides in distinct classes of neurons in the myenteric and submucosal plexuses (Costa *et al*., 1996; Furness, 2000) and the expression of opioid receptors by enteric neurons and intestinal muscle cells. When released from neurons, opioid peptides are likely to play a transmitter role in the enteric regulation of propulsive motility. Thus, blockade of opioid receptors by naloxone facilitates intestinal peristalsis and rescues peristaltic motor activity from blockade by atropine or hexamethonium (Kromer & Schmidt, 1982; Holzer *et al*., 1998). The inhibitory effect of opioid receptor agonists on motility is thought to arise primarily from interruption of transmission within enteric nerve pathways governing muscle contraction (Tonini *et al*., 1992). Transmission is blocked *via* a presynaptic site of action, whereby the release of acetylcholine and other excitatory transmitters is attenuated, although postsynaptic effects have also been described (Cherubini *et al*., 1985). In addition, opiates are known to contract intestinal muscle, an action that may be due to facilitation of acetylcholine release from some excitatory enteric neurons (Giuliani *et al*., 1996), depression of nitric oxide release from inhibitory enteric neurons (Lenard *et al*., 1999), or direct activation of muscle cells that express opioid receptors (Bitar & Makhlouf, 1985; Hirning *et al*., 1985; Brown *et al*., 1998). Induction of stationary segmentations combines with inhibition of peristalsis and depression of secretory activity to bring about constipation (Kromer, 1993; de Luca & Coupar, 1996).

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The nature of the opioid receptors, whereby opiates block gastrointestinal propulsion, is still a matter of dispute, although the discrepancies may, in part, reflect species differences. Studies with isolated tissues from the human intestine suggest that δ-, κ- and μ-opioid (OP_1, OP_2 and OP_3) receptors contribute to opiate-induced inhibition of motor activity (Bauer *et al*., 1991; Chamouard *et al*., 1993). Likewise, δ-, κ- and μ-opioid receptor agonists have been found to modify the ascending motor reflex in the rat intestine (Allescher *et al*., 2000), whereas propulsive peristalsis in this species is inhibited by δ- and μ-, but not κ-, opioid receptor agonists (Tavani *et al*., 1984; Coupar, 1995). Conversely, in the canine small intestine it is κ-opioid receptors that play a predominant role in the opiate-induced inhibition of myoelectrical activity (Telford *et al*., 1988), while μ-, but not κ-, opioid receptors contract the muscle (Hirning *et al*., 1985). Only δ- and κ-opioid receptors are expressed by enteric neurons of the porcine ileum, and functional studies indicate that these two receptors bring about the antiperistaltic and antisecretory actions of opiates in the porcine gut (Brown *et al*., 1998; Poonyachoti *et al*., 2001). Myenteric neurons in the guinea-pig intestine express a considerable number of μ-opioid receptors (Sternini *et al*., 1996), but the opiate-induced inhibition of cholinergic transmission is mediated by both μ- and κ-opioid receptors (Cherubini & North, 1985; Kojima *et al*., 1994). In contrast, the role of δ-opioid receptors in the regulation of guinea-pig intestinal motility is unclear. While δ-opioid receptor agonists attenuate neurogenic contractions in the guinea-pig isolated colon (Giuliani *et al*., 1996), they are rather inactive in the ileum (Johnson *et al*., 1987), although they modify propulsive peristalsis to some extent (Waterman *et al*., 1992).

Apart from species differences, some of the diverse observations regarding the opioid receptor types relevant to the antiperistaltic action of opiates may also be due to the limited receptor selectivity of some of the agonists and antagonists employed. We therefore set out to perform a systematic pharmacological study of the effects of selective opioid receptor agonists and antagonists on propulsive peristalsis in the guinea-pig isolated small intestine. The first aim of the current work was to characterize the antiperistaltic motor effects of a range of opiates in clinical use as well as of δ-, κ- and μ-opioid receptor-selective agonists. Secondly, we went on to check the receptor preference of some of the agonists under study by δ-, κ- and μ-opioid receptor-selective antagonists and thus to obtain more reliable information on the opioid receptor types involved in peristaltic motor regulation. The third aim was to address the ability of δ-, κ- and μ-opioid receptor-selective antagonists to modify intestinal peristalsis and thereby to explore which receptors may be targeted by endogenous opioid peptides during the physiological control of peristalsis.

Methods

Recording of peristalsis

The small intestine (jejunum and ileum) of adult guinea-pigs (TRIK strain, either sex, 350–450 g body weight) was isolated, flushed of luminal contents and placed, for up to 4 h, in Tyrode solution kept at room temperature and oxygenated with a mixture of 95% O_2 and 5% CO_2 (Heinemann *et al*., 1999). The composition of the Tyrode solution was (mM): NaCl 136.9, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.0, $NaHCO_3$ 11.9, NaH_2PO_4 0.4 and glucose 5.6. The small intestine (jejunum and ileum) was divided into eight segments, each being approximately 10 cm long. Since the baseline peristaltic parameters recorded here (see below) did not significantly differ between segments taken from the proximal jejunum and distal ileum ($n=9$), the segments were assigned randomly to the pharmacological treatments under study. Four intestinal segments were set up in parallel and secured horizontally in organ baths containing 30 ml of Tyrode solution at 37°C. In order to elicit propulsive peristalsis, prewarmed Tyrode solution was continuously infused into the lumen of the segments at a rate of 0.5 ml min^{-1} (Heinemann *et al*., 1999). The intraluminal pressure at the aboral end of the segments was measured with a pressure transducer whose signal was, *via* an analogue/digital converter, fed into a personal computer and recorded and analysed with the software Peristal 1.0 (Heinemann *et al*., 1999).

The fluid passing through the gut lumen was directed into a vertical outlet tubing which ended 4 cm above the fluid level in the organ bath. When fluid was infused, the intraluminal pressure rose slowly until it reached a threshold at which peristalsis was triggered (Figure 1). The aborally moving wave of peristaltic contraction (peristaltic wave) resulted in a spike-like increase in the intraluminal pressure, which caused emptying of the segment if the maximal pressure of the peristaltic wave exceeded the level of 400 Pa as set by the position of the outlet tubing.

Experimental protocol

The preparations were allowed to equilibrate in the organ bath for a period of 30 min during which they were kept in a quiescent state. Thereafter the bath fluid was renewed and peristaltic motility initiated by intraluminal perfusion of the segments. After basal peristaltic activity had been recorded for a 30 min period, the drugs to be tested were added to the bath, i.e., to the serosal surface of the intestinal segments, at volumes not exceeding 1% of the bath volume. The corresponding vehicle solutions were devoid of any effect.

Two sets of experiments were performed. Firstly, the peristaltic motor effects of the opioid receptor agonists morphine (0.01–111.1 μM), DAMGO (3–443 nM), fentanyl (0.1–100 nM), hydromorphone (0.001–1 μM), codeine (0.1–100 μM), loperamide, 14-methoxymetopon (1–111 nM), ICI-204,448, BRL-52,537 (0.3–44.3 nM) and SNC-80 (0.3–14.3 μM) were studied. The receptor agonists were added to the bath in a cumulative manner at 15 min intervals. Secondly, the ability of cyprodime, naltrindole, nor-binaltorphimine and HS-378 to influence the antiperistaltic activity of morphine, DAMGO, ICI-204,448, BRL-52,537 and SNC-80 was examined. In this type of experiment the intestinal segments were first exposed to vehicle, cyprodime (10 μM), naltrindole, nor-binaltorphimine (30 nM) or HS-378 (1 μM) for 30 min, whereafter morphine (0.01–111.1 μM), DAMGO (3–443 nM), ICI-204,448, BRL-52,537 (0.3–44.3 nM) or SNC-80 (0.3–14.3 μM) was added to the bath in a cumulative manner at 15 min intervals. Each protocol was carried out with at least six segments from six different guinea-pigs.

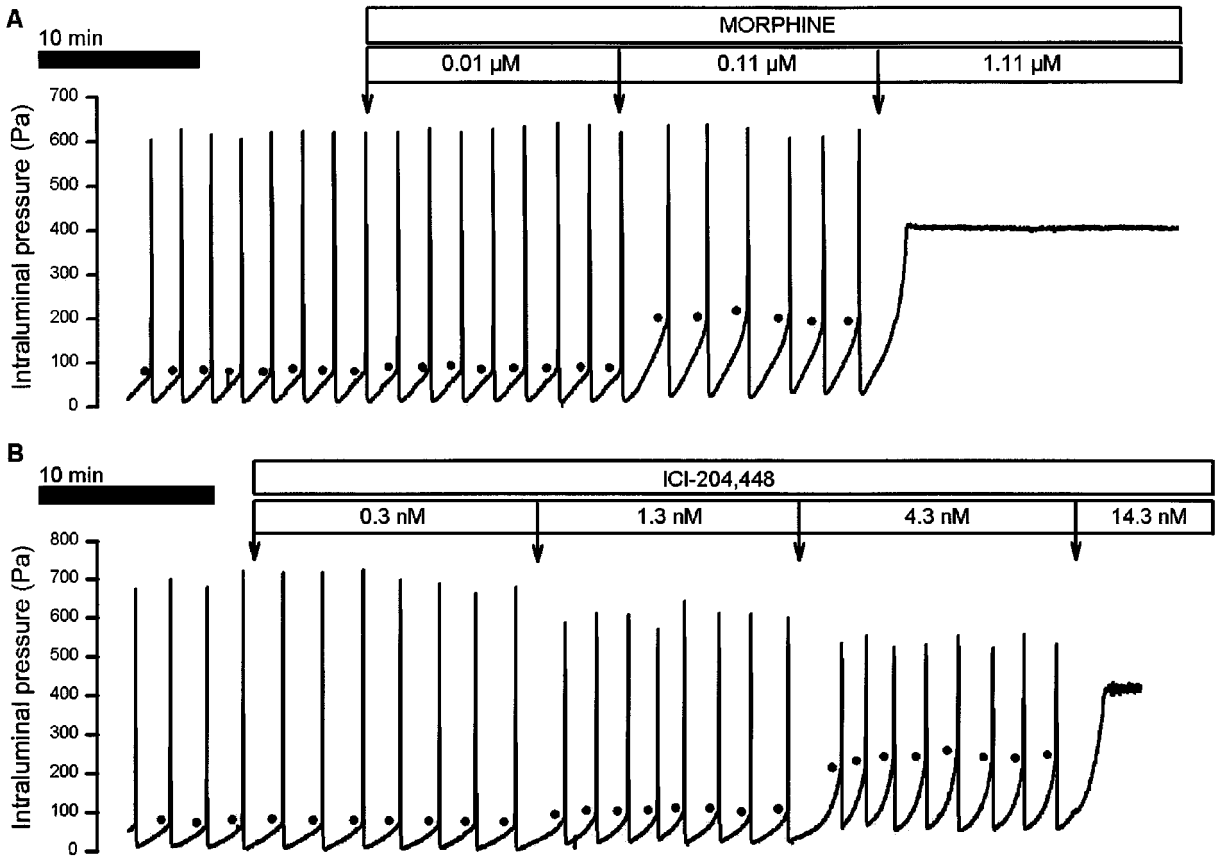

Figure 1 Recordings of the effect of morphine (A) and ICI-204,448 (B) on peristalsis. The drugs were administered to the organ bath at the specified concentrations. As can be seen, morphine and ICI-204,448 inhibited peristalsis by increasing the peristaltic pressure threshold (indicated by dots); ICI-204,448 also reduced the amplitude of the peristaltic waves.

Evaluation of peristalsis

The recordings of peristalsis were analysed with the software 'Peristal 1.0' with regard to four different parameters: the peristaltic pressure threshold (PPT), the residual baseline pressure, the amplitude (maximal pressure) of the peristaltic waves and the maximal acceleration of the peristaltic waves (Shahbazian *et al*., 2001). PPT (Pa) is the intraluminal pressure at which a peristaltic wave is triggered. Inhibition of peristalsis was associated with an increase in PPT, and abolition of peristalsis manifested itself in a lack of propulsive motility in spite of an intraluminal pressure of 400 Pa. Although in this case PPT exceeded 400 Pa, abolition of peristalsis was expressed quantitatively by assigning PPT a value of 400 Pa in order to obtain numerical results suitable for further statistical evaluation. The residual baseline pressure (Pa) equals the minimal intraluminal pressure that is achieved after completion of each peristaltic wave and thus reflects a sensitive measure of the emptying capacity of the peristaltic waves (Shahbazian *et al*., 2001). Further indices of peristaltic effectiveness are the amplitude of the peristaltic waves (Pa) and the maximal acceleration of the peristaltic waves (Pa s^{-2}), which is determined not only by the speed with which the muscle contracts but also by the speed with which the contraction moves aborally to empty the segments.

The effects of opioid receptor agonists on peristalsis were quantified such that peak changes of the peristaltic parameters occurring during the 15 min observation periods were analysed. To this end, the peristaltic parameters of 3–4 peristaltic waves at the peak effect were averaged. Analogously, the influence of opioid receptor antagonists on peristalsis was evaluated by calculating the mean parameters of 3–4 peristaltic waves determined 30 min after antagonist administration. The baseline values were measured by averaging the parameters of 3–4 peristaltic waves recorded immediately before administration of vehicle, antagonist or agonist. Although all four peristaltic parameters were recorded and calculated in all experiments, PPT proved to be the prime parameter reflecting the peristaltic motor effect of opioid receptor ligands. Hence, the results of the majority of the experiments are described in terms of PPT changes only.

Drugs and solutions

The sources of the drugs used here were as follows: BRL-52,537 ((±)-1-(3,4-dichlorophenyl) acetyl-2-(1-pyrrolidinyl) methylpiperidine hydrochloride), DAMGO (Tyr-D-Ala-Gly-NMe-Phe-Gly-ol), ICI-204,448 ((R,S)-N-[2-(N-methyl-3,4-dichlorophenylacetamido) - 2 - (3 - carboxyphenol) - ethyl]pyrrolidine hydrochloride), naltrindole hydrochloride and nor-binaltorphimine dihydrochloride (nor-BNI), SNC-80 ((+)-4-(alphaR)-alpha-((2R, 5R)-4-allyl-2,5-dimethyl - 1-piperazinyl)-

3-methoxybenzyl]-N,N-diethylbenzamide) were obtained from Tocris Cookson (Bristol, U.K.). Cyprodime, HS-378 ((-)-17-(cyclopropylmethyl) - 6,7 - didehydro - 4,5α -epoxy-14β- ethoxy-5β-methylindolo[2',3':6,7]morphinan-3-ol hydrochloride) and 14-methoxymetopon were synthesized at the Department of Pharmacy, Division of Pharmaceutical Chemistry, of the University of Innsbruck (Austria). Fentanyl citrate was provided by Janssen Pharmaceutica (Beerse, Belgium) and hydromorphone hydrochloride by Napp Laboratories (Cambridge, U.K.), while morphine hydrochloride and codeine phosphate hemihydrate were obtained from Endo Laboratories (Richmond Hill, N.Y., U.S.A.). The drugs were dissolved with appropriate media, the concentrations given hereafter in parenthesis referring to the stock solutions. Cyprodime, SNC-80 (10 mM) and HS-378 (1 mM) were dissolved in dimethyl sulphoxide. Fentanyl, hydromorphone, ICI-204,448, morphine, naltrindole (1 mM), BRL-52,537 (5 mM), 14-methoxymetopon (10 mM) and codeine (100 mM) were dissolved in distilled water, nor-binaltorphimine (1 mM) in Tyrode solution and DAMGO (10 mM) in 0.1 M acetic acid. These stock solutions were diluted with Tyrode solution as required, except for cyprodime and HS-378 which were diluted with dimethyl sulphoxide. Care was taken that none of the organic solvents reached concentrations higher than 0.1% in the bathing solution.

Statistics

Quantitative data are presented as means + or ± s.e.mean of n experiments, n referring to the number of guinea-pigs used in the test. The results were evaluated with the paired t-test, two sample t-tests, one-way analysis of variance (ANOVA) for repeated measures or one-way ANOVA followed by Dunnett's test, as appropriate. Probability values of $P<0.05$ were regarded as significant. The opioid receptor agonist concentrations (EC_{50}) that caused a half-maximal increase in PPT were extrapolated from the respective part of the individual concentration–response curves. In select instances, the antagonist affinity of cyprodime (10 μM) was expressed by the apparent K_B value which was estimated according to the equation

$$pk_B = \log[\text{dose ratio} - 1] - \log[\text{antagonist concentration}]$$

(Jenkinson, 1991).

The dose ratio was determined by extrapolation of the agonist concentrations that caused 30% of the maximal response in the presence and absence of cyprodime, respectively (Patacchini *et al*., 1997).

Results

Effects of μ-opioid receptor agonists on peristalsis

Quantitative estimates of the peristalsis parameters at baseline were: PPT, 69±2.6 Pa; residual baseline pressure, 12±1.1 Pa; maximal pressure of the peristaltic waves, 722±18 Pa; and maximal acceleration of the peristaltic waves, 376±15 Pa s^{-2} ($n=94$). Administration of the μ-opioid receptor agonist morphine (0.01–1.1 μM) to the organ bath caused a concentration-dependent inhibition of intestinal peristalsis (Figure 1A). Thus, when the opioid concentration in the bath was cumulatively increased, morphine gradually enhanced PPT, lowered the frequency of the peristaltic waves and, at concentrations between 1.1 and 11.1 μM, invariably abolished peristaltic motor activity (Figures 1A and 2). The concentration-related increase in PPT was accompanied by an increase in the residual baseline pressure and a decrease in the maximal acceleration of the peristaltic waves, whereas the maximal pressure of the peristaltic waves was not significantly altered (Figures 1A and 2).

The inhibitory effect of morphine on peristalsis was mimicked by other μ-opioid receptor agonists in clinical use (loperamide, hydromorphone, fentanyl and codeine) as well as by 14-methoxymetopon and the enkephalin derivative DAMGO (Figures 1B, 3 and 4). While, qualitatively, the antiperistaltic action of these μ-opioid receptor agonists did not differ from that of morphine (Figure 1A,B), their potencies in enhancing PPT varied to a large extent (Figure 3). Extrapolation of the average agonist concentrations that caused a half-maximal increase in PPT (EC_{50}, means±s.e.-mean given in brackets, significant differences estimated by ANOVA plus Dunnett's test) showed that the rank order of potency was fentanyl (2.4±1.2 nM), 14-methoxymetopon (9.9±3.1 nM), loperamide (21±4.3 nM, $P<0.05$ *versus* fentanyl), hydromorphone (26±3.2 nM, $P<0.05$ *versus* fentanyl), DAMGO (100±18 nM, $P<0.05$ *versus* hydromorphone), morphine (0.65±0.23 μM, $P<0.05$ *versus* DAMGO) and codeine (21±3.8 μM, $P<0.05$ *versus* morphine).

Effects of κ-opioid receptor agonists on peristalsis

Addition of the κ-opioid receptor agonists ICI-204,448 and BRL-52,537 (0.3–44.3 nM) to the organ bath enhanced PPT in a concentration-dependent manner (Figures 1B, 2 and 5). At the highest concentrations tested, peristaltic motility was completely arrested. The antiperistaltic action of these agonists was qualitatively similar to that of the μ-opioid receptor agonists, as can be seen from Figure 1B which shows a recording of the peristaltic motor response to ICI-204,448. Quantitative analysis of the peristaltic motor effect brought about by ICI-204,448 revealed that, in addition to a decrease in the maximal acceleration of the peristaltic waves and an increase in PPT and the residual baseline pressure, this κ-opioid receptor agonist also lowered the maximal pressure of the peristaltic waves, which differentiates its effect from that of morphine (Figures 1B and 2).

Effects of δ-opioid receptor agonists on peristalsis

Exposure of the intestinal segments to increasing concentrations of the δ-opioid receptor agonist SNC-80 (0.3–14.3 μM) suppressed peristalsis (Figures 2 and 6). Quantitative analysis of the effect of SNC-80 showed that PPT and the residual baseline pressure were gradually increased while the maximal pressure and the maximal acceleration of the peristaltic waves was decreased (Figure 2). In addition, it appeared as if the concentration–response curve for the effect of SNC-80 to enhance PPT was steeper than the respective curve for morphine and ICI-204,448 (Figures 2 and 6).

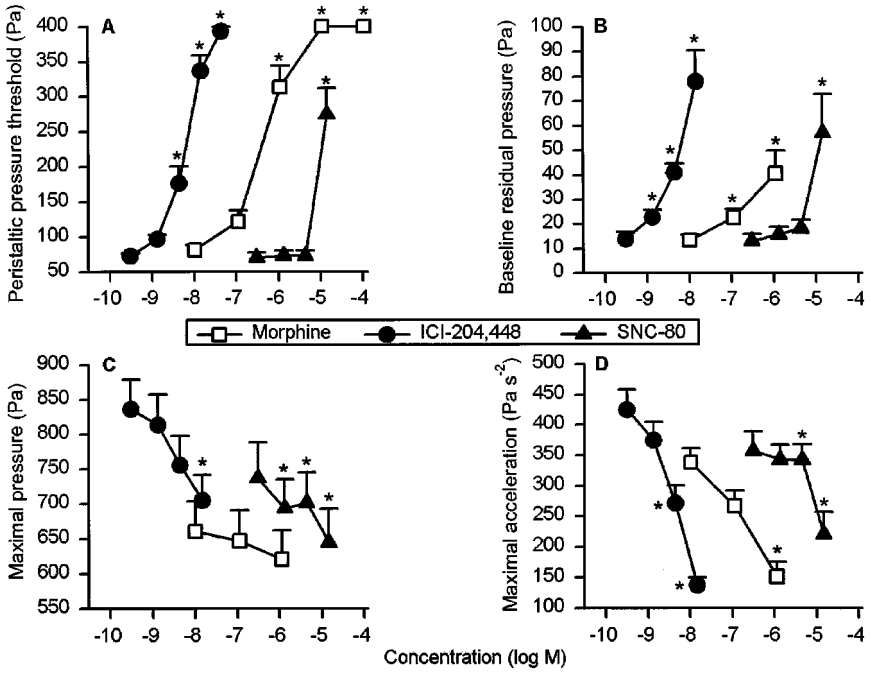

Figure 2 Concentration–response relationship for the effects of morphine, ICI-204,448 and SNC-80 to enhance the peristaltic pressure threshold (A) and the residual baseline pressure (B) and to reduce the amplitude (maximal pressure; C) and the maximal acceleration (D) of the peristaltic waves. The concentration–response curves were recorded in a cumulative manner at 15-min intervals. The values represent means+s.e.mean; $n=13$ for morphine, $n=16$ for ICI-204,448, $n=17$ for SNC-80. $*P<0.05$ *versus* parameters recorded immediately before drug administration (ANOVA for repeated measures followed by Dunnett's test).

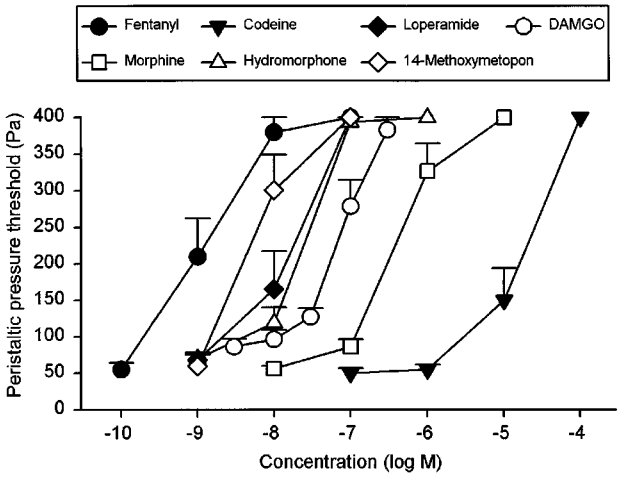

Figure 3 Concentration–response relationship for the effects of fentanyl, morphine, codeine, hydromorphone, loperamide, 14-methoxymetopon and DAMGO to enhance the peristaltic pressure threshold. The concentration–response curves were recorded in a cumulative manner at 15-min intervals. The values represent means+s.e.mean; $n=7$ for fentanyl; $n=7$ for morphine, $n=7$ for codeine, $n=5$ for hydromorphone, $n=6$ for loperamide, $n=8$ for 14-methoxymetopon, $n=8$ for DAMGO.

Effects of μ-, κ- and δ-opioid receptor antagonists on peristalsis

Addition of the μ-opioid receptor antagonist cyprodime (10 μM) and the κ-opioid receptor antagonist nor-binaltorphimine (30 nM) led to a prompt and sustained stimulation of peristaltic motor activity as deduced from a decrease in PPT seen 30 min later (Table 1). In contrast, the δ-opioid receptor antagonists naltrindole (30 nM) and HS-378 (1 μM) failed to alter PPT to any significant degree (Table 1).

Effects of opioid receptor antagonists on the antiperistaltic action of μ-opioid receptor agonists

In order to examine which opioid receptors mediate the peristaltic motor inhibition caused by morphine and DAMGO, concentration–response curves for the effect of these agonists to enhance PPT were recorded 30 min after exposure of the intestinal segments to a μ-, κ- or δ-opioid receptor antagonist (Figure 4). Compared with vehicle, cyprodime (10 μM) completely prevented the antiperistaltic action of morphine (0.01–1.11 μM) and DAMGO (3–443 nM) and significantly depressed the effect of 111 μM morphine (Figure 4). The apparent K_B value of cyprodime *versus* morphine was found to be 79 ± 35 nM ($n=8$). Naltrindole (30 nM) failed to alter the peristaltic motor inhibition caused by morphine and DAMGO, whereas HS-378 (1 μM) caused some rightward shift of the concentration–response curve for both μ-opioid receptor agonists (Figure 4). While the antiperistaltic action of morphine was left unchanged by nor-binaltorphimine (30 nM), that of DAMGO was reduced to a significant extent (Figure 4).

Effects of opioid receptor antagonists on the antiperistaltic action of κ-opioid receptor agonists

The antiperistaltic motor action of the preferential κ-opioid receptor agonists ICI-204,448 and BRL-52,537 (0.3–44.3 nM)

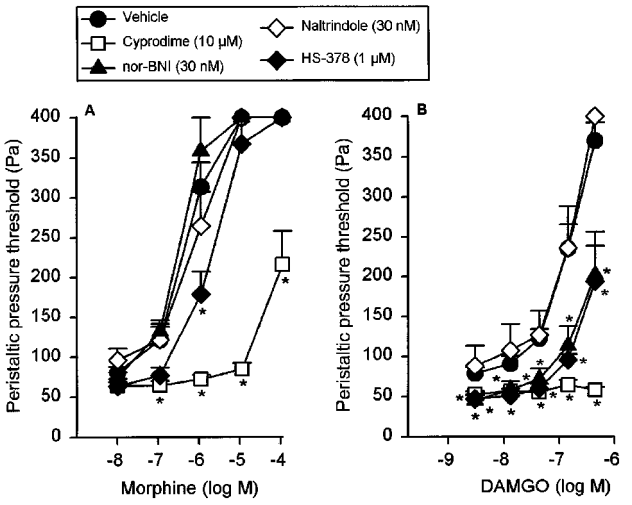

Figure 4 Effects of morphine (A) and DAMGO (B) to increase the peristaltic pressure threshold as recorded in the presence of vehicle, cyprodime, nor-binaltorphimine (nor-BNI), naltrindole and HS-378. The antagonists were administered 30 min before addition of the agonists. The concentration–response curves were recorded in a cumulative manner at 15-min intervals. The values represent means+s.e.mean; $n=13$ for vehicle/morphine, $n=17$ for vehicle/DAMGO, $n=8$ for cyprodime/morphine, $n=7$ for HS-378/morphine, $n=5$ for naltrindole/morphine, $n=6$ for all other treatments. $^*P<0.05$ *versus* effect of respective agonist concentration recorded in the presence of vehicle (ANOVA followed by Dunnett's test).

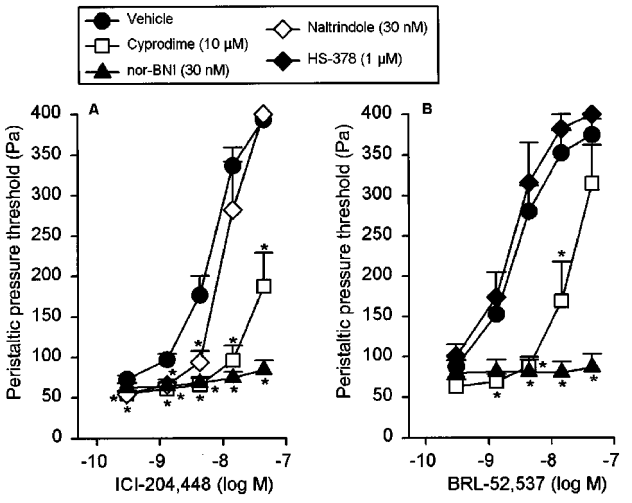

Figure 5 Effects of ICI-204,448 (A) and BRL-52,537 (B) to increase the peristaltic pressure threshold as recorded in the presence of vehicle, cyprodime, nor-binaltorphimine (nor-BNI), naltrindole and HS-378. The antagonists were administered 30 min before addition of the agonists. The concentration–response curves were recorded in a cumulative manner at 15-min intervals. The values represent means+s.e.mean; $n=16$ for vehicle/ICI-204,448, $n=15$ for vehicle/BRL-52,537, $n=7$ for all other treatments. $^*P<0.05$ *versus* effect of respective agonist concentration recorded in the presence of vehicle (ANOVA followed by Dunnett's test).

was completely suppressed by nor-binaltorphimine (30 nM) when compared with the effects of these agonists recorded in the presence of vehicle (Figure 5). In addition, cyprodime (10 μM) led to a significant rightward shift of the concentration–response curves for the two κ-opioid receptor agonists.

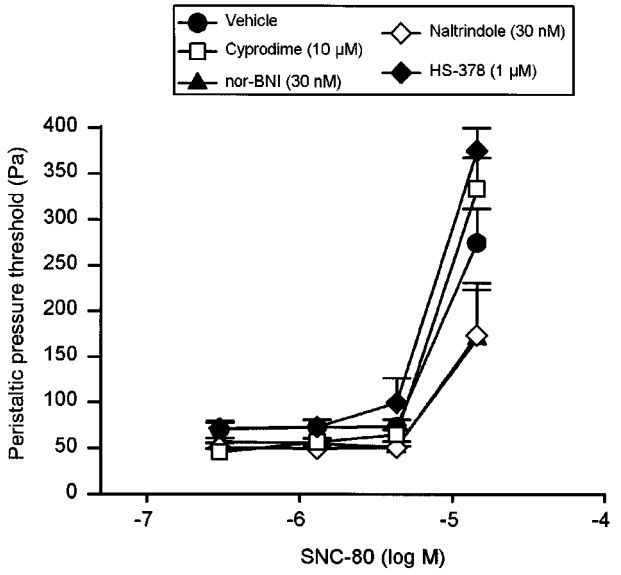

Figure 6 Effects of SNC-80 to increase the peristaltic pressure threshold as recorded in the presence of vehicle, cyprodime, nor-binaltorphimine (nor-BNI), naltrindole and HS-378. The antagonists were administered 30 min before addition of the agonist. The concentration–response curves were recorded in a cumulative manner at 15-min intervals. The values represent means+s.e.mean; $n=17$ for vehicle, $n=7$ for cyprodime, $n=6$ for all other treatments.

The apparent K_B values of cyprodime *versus* ICI-204,448 and BRL-52,537 were calculated to be 2.85 ± 2.09 μM ($n=7$) and 5.27 ± 2.40 μM ($n=5$), respectively. Naltrindole (30 nM) and HS-378 (1 μM) failed to influence the inhibitory motor effect of ICI-204,448 and BRL-52,537, respectively (Figure 5).

Effects of opioid receptor antagonists on the antiperistaltic action of δ-opioid receptor agonists

The peristaltic motor inhibition brought about by the δ-opioid receptor agonist SNC-80 (0.3–14.3 μM) was not significantly altered by any of the opioid receptor antagonists under study (Figure 6). Thus, the concentration–response curves for the effect of SNC-80 to enhance PPT did not differ when they were recorded in the presence of vehicle, naltrindole (30 nM), HS-378 (1 μM), cyprodime (10 μM) or nor-binaltorphimine (30 nM).

Discussion

The major results of this study are as follows: (1) Agonists at μ-, κ- and δ-opioid receptors inhibit propulsive peristalsis in the guinea-pig small intestine; (2) Agonists at μ-, κ- and δ-opioid receptors differ in their mode of action since, unlike a κ-opioid and δ-opioid receptor agonist, a μ-opioid receptor agonist fails to depress the amplitude of the peristaltic waves; (3) The antiperistaltic action of μ-opioid receptor agonists is preferentially mediated by μ-opioid receptors while that of κ-opioid receptor agonists is predominantly brought about by κ-opioid receptors; (4) The antiperistaltic action of a δ-opioid receptor agonist seems to be unrelated to opioid receptor activation; and (5) Endogenous opioid peptides dampen peristalsis *via* activation of μ- and κ-, but not δ-, opioid receptors.

Table 1 Effect of opioid receptor antagonists on the peristaltic pressure threshold (PPT)

Treatment	n	*PPT before treatment* (Pa)	*PPT after treatment* (Pa)	P
Vehicle	94	69±2.6	71±2.4	n.s.
Cyprodime (10 μM)	36	65±3.0	51±1.9	<0.01
nor-BNI (30 nM)	32	70±4.3	61±4.7	<0.01
Naltrindole (30 nM)	23	64±4.9	60±5.3	n.s.
HS-378 (1μM	26	72±5.4	71±5.2	n.s.

PPT was determined immediately before and 30 min after administration of the opioid receptor antagonists or their vehicle to the organ bath. The data given are means±s.e.mean, the P values being calculated with the paired *t*-test. nor-BNI, nor-binaltorphimine; n.s., not significant.

Peristaltic motor inhibition caused by μ-opioid receptor agonists

DAMGO (Handa *et al*., 1981), 14-methoxymetopon (Schmidhammer *et al*., 1990) and a range of μ-opioid receptor-preferring agonists in clinical use (morphine, fentanyl, loperamide, hydromorphone and codeine) inhibited peristalsis in a concentration-related manner. While their efficacy (abolition of peristalsis) was identical, their potencies in depressing peristalsis differed widely. On average, fentanyl was 268 times, 14-methoxymetopon 66 times, loperamide 32 times, hydromorphone 25 times and DAMGO 6.5 times more potent than morphine, whereas codeine was 36 times less active than morphine. This order of potency in suppressing peristalsis is in overall agreement with the compounds' order of affinity for μ-opioid receptors and their order of analgesic potency (Handa *et al*., 1981; Schmidhammer *et al*., 1990; Dhawan *et al*., 1996). When such orders of relative potency are compared with each other it need be considered that the relative potency of the μ-opioid receptor agonists under study will also be influenced by their pharmacokinetic behaviour in our *in vitro* preparation where compounds administered into the organ bath need to penetrate the serosa and longitudinal muscle before they reach the myenteric plexus and circular muscle. In addition, it need be taken into account that the peristalsis-modifying effect of opioid receptor ligands reflects their net action on multiple opioid receptors at multiple sites of the nerve–muscle system subserving peristalsis. It is nevertheless evident from the current data that the antiperistaltic activity of μ-opioid receptor agonists parallels their analgesic activity and that hence an improvement of the adverse effect profile of μ-opioid receptor agonists on propulsive motility may be achieved only by modification of their pharmacokinetic, not pharmacodynamic, properties.

The finding that all μ-opioid receptor agonists under study enhanced PPT indicates that they made the neural circuits subserving peristalsis less sensitive to distension. In addition, they enhanced the residual baseline pressure and attenuated the maximal acceleration of the peristaltic waves, while the maximal amplitude of the peristaltic waves remained unchanged, which reflects some diminution of peristaltic effectiveness. These characteristics of the antiperistaltic action of μ-opioid receptor agonists are consistent with the abundant expression of μ-opioid receptors on myenteric neurons of the guinea-pig intestine (Sternini *et al*., 1996) and with the ability of μ-opioid receptor agonists to interrupt cholinergic and non-cholinergic transmission at multiple sites within the enteric nervous system (Cherubini *et al*., 1985; Johnson *et al*., 1988; Tonini *et al*., 1992; Lenard *et al*., 1999).

The opioid receptors mediating the antiperistaltic action of morphine and DAMGO were analysed with the use of the opioid receptor subtype-selective antagonists cyprodime (Schmidhammer *et al*., 1989), nor-binaltorphimine (Takemori *et al*., 1988), naltrindole (Portoghese *et al*., 1988) and HS-378 (Spetea *et al*., 2001). The μ-opioid receptor-selective antagonist cyprodime (K_i=5.4 nM) shows a κ/μ selectivity of 405 and a δ/μ selectivity of 45 (Marki *et al*., 1999). For the κ-opioid receptor-selective antagonist nor-binaltorphimine (K_i=0.28 nM) a μ/κ selectivity of 169 and a δ/κ selectivity of 153 has been reported (Takemori *et al*., 1988). Naltrindole is a δ-opioid receptor-selective antagonist (K_i=0.14 nM) with a μ/δ selectivity of 93 and a κ/δ selectivity of 113, while HS-378 is a δ-opioid receptor-selective antagonist (K_i=0.78 nM) with improved δ preference, its μ/δ selectivity being 436 and its κ/δ selectivity being 172 (Spetea *et al*., 2001). As expected, the μ-opioid receptor-selective antagonist cyprodime at the concentration of 10 μM completely prevented morphine and DAMGO from inhibiting peristalsis, which is consistent with its high affinity for μ-opioid receptors (Marki *et al*., 1999). The δ-opioid receptor-selective antagonist naltrindole (30 nM) failed to alter the antiperistaltic action of morphine and DAMGO, whereas the δ-opioid receptor-selective antagonist HS-378, at the concentration of 1 μM, caused a slight but significant rightward shift of the morphine and DAMGO concentration–response curves. This effect of HS-378 (K_i=0.34 μM at μ-opioid receptors) can be explained by its μ-opioid receptor antagonistic activity at the concentration of 1 μM (Spetea *et al*., 2001).

The κ-opioid receptor antagonist nor-binaltorphimine (K_i=47 nM at μ-opioid receptors; Takemori *et al*., 1988), tested at the concentration of 30 nM (Berzetei-Gurske & Toll, 1992), shifted the DAMGO concentration–response curve to the right but left the action of morphine unaltered. Although the differential antagonism of DAMGO and morphine by nor-binaltorphimine suggests that DAMGO inhibits peristalsis partly by activation of κ-opioid receptors, this inference is negated by the high μ-/κ-opioid receptor selectivity of this enkephalin analogue (Handa *et al*., 1981; Corbett *et al*., 1984). Since the ability of DAMGO to enhance noradrenaline release from the guinea-pig colon is antagonized both by naloxone and nor-binaltorphimine (Cosentino *et al*., 1997), it appears conceivable that μ- and κ-opioid receptors undergo a functional interaction that is differentially addressed by morphine and DAMGO. The likely co-expression of μ- and κ-opioid receptors by the same myenteric neurons (Kojima *et al*., 1994; Cosentino *et al*., 1995; Abalo *et al*., 2000) even provokes the speculation that μ- and κ-opioid receptors form heterodimers with novel pharmacological properties, as has

been found for μ- and δ-opioid receptors (Gomes *et al*., 2000).

Peristaltic motor inhibition caused by κ-opioid receptor agonists

The two κ-opioid receptor-selective agonists used here, ICI-204,448 (Shaw *et al*., 1989) and BRL-52,537 (Vecchietti *et al*., 1991), inhibited peristalsis in a manner that was largely indistinguishable from that of morphine and other μ-opioid receptor agonists. The only difference was that, unlike morphine, ICI-204,448 also reduced the amplitude of the peristaltic waves, which points to a pronounced depression of peristaltic effectiveness. Speculatively, this difference may be due to the fact that μ-, but not κ-, opioid receptor agonists can lead, through a variety of mechanisms, to contraction of intestinal muscle (Bitar & Makhlouf, 1985; Hirning *et al*., 1985; Giuliani *et al*., 1996; Lenard *et al*., 1999). If so, the increase in muscle tone brought about by morphine may to some extent counterbalance the morphine-induced inhibition of the neural circuits subserving peristalsis, whereas the inhibitory action of ICI-204,448 on the enteric peristaltic pathways is not ameliorated by any contractile action of the compound. Thus, the results obtained with ICI-204,448 and BRL-52,537 illustrate that κ-opioid receptor agonists are highly potent and efficacious in suppressing intestinal peristalsis, an adverse effect that is likely to confound the utility of this class of drugs as much as that of μ-opioid receptor agonists.

Pharmacological analysis with opioid receptor subtype-selective antagonists corroborated that the antiperistaltic action of ICI-204,448 and BRL-52,537 is predominantly mediated by κ-opioid receptors, because it was abolished by nor-binaltorphimine. While the δ-opioid receptor antagonists naltrindole and HS-378 failed to influence the peristaltic motor inhibition caused by ICI-204,448 and BRL-52,537, respectively, the μ-opioid receptor antagonist cyprodime (10 μM) caused a rightward shift of the concentration–response curves for both κ-opioid receptor agonists. This inhibitory effect of cyprodime reflects blockade of κ-opioid receptors because (i) ICI-204,448 and BRL-52,537 are devoid of μ-opioid receptor-agonistic activity at the concentrations employed here (Shaw *et al*., 1989; Vecchietti *et al*., 1991) and (ii) the K_B values of cyprodime for preventing the antiperistaltic action of ICI-204,448 (2.85 μM) and BRL-52,537 (5.27 μM) are in the same order of magnitude as the K_i of cyprodime (2.19 μM) for inhibiting κ-opioid receptor binding (Marki *et al*., 1999). We therefore conclude that the antiperistaltic action of ICI-204,448 and BRL-52,537 in the guinea-pig small intestine is exclusively mediated by κ-opioid receptors.

Peristaltic motor inhibition caused by δ-opioid receptor agonists

Although SNC-80 (Bilsky *et al*., 1995) led to a concentration-dependent suppression of peristalsis, we put forward a number of reasons that the action of this δ-opioid receptor agonist is not brought about by δ-opioid receptors. (i) The concentration–response curve for the ability of SNC-80 to increase PPT was unusually steep when compared with that of μ- and κ-opioid receptor agonists. (ii) Although low nanomolar concentrations of SNC-80 activate δ-opioid receptors (Bilsky *et al*., 1995), micromolar concentrations of SNC-80 were needed to impair peristaltic performance (this study) and to depress electrically evoked contractions of the guinea-pig ileum (Bilsky *et al*., 1995). (iii) Since neither naltrindole and HS-378 nor cyprodime and nor-binaltorphimine were able to significantly modify the peristaltic motor inhibition caused by SNC-80, it would appear that this compound suppressed peristaltic motility by an action unrelated to δ-, μ- and κ-opioid receptors.

The inference that δ-opioid receptors do not participate in the antiperistaltic action of opioid receptor agonists in the guinea-pig small intestine is in line with other reports that δ-opioid receptors are functionally absent from this tissue or, at best, play only a minor role (Johnson *et al*., 1987; Waterman *et al*., 1992; Bilsky *et al*., 1995; Dhawan *et al*., 1996). This view is further supported by the failure of δ-opioid receptor antagonists to modify peristalsis.

Peristaltic motor stimulation by opioid receptor antagonists

It has previously been noted that naloxone (0.5 μM) lowers PPT and thereby facilitates peristalsis in the guinea-pig small intestine (Holzer *et al*., 1998). The present study revealed that both μ- and κ-opioid receptors contribute to this action, given that the properistaltic effect of naloxone is shared by cyprodime and nor-binaltorphimine, but not naltrindole and HS-378. It thus seems as if endogenous opioid peptides released in the course of propulsive motility dampen peristaltic performance *via* activation of μ- and κ-opioid receptors. Consistent with this inference is the ability of both cyprodime and nor-binaltorphimine to enhance the release of acetylcholine from the myenteric plexus (Cosentino *et al*., 1995). The peristaltic motor actions of opioid receptor-selective antagonists are complementary to those of opioid-receptor-selective agonists and corroborate the view that the neural circuits controlling peristalsis in the guinea-pig small intestine are under the influence of μ- and κ-, but not δ-, opioid receptors. This view concurs with the observations of Culpepper-Morgan *et al*. (1988) that opiates slow gastrointestinal transit in the guinea-pig *via* activation of μ- and κ-opioid receptors.

In a clinical perspective it would appear that removal of opioidergic blockade of transmission in enteric motor pathways by appropriate opioid receptor antagonists is an effective way to stimulate peristaltic motor activity (this study) and to rescue propulsive motility from shutdown by acetylcholine receptor antagonists (Holzer *et al*., 1998). This hypothesis implies that opioid receptor antagonists that target peristalsis-relevant opioid receptors in the enteric nervous system may be used as prokinetics, both in the prevention of postoperative ileus (Taguchi *et al*., 2001) and in the treatment of other pathological states of propulsive motor inhibition.

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References

ABALO, R., PASCUAL, D., GOICOECHEA, C. & MARTIN, M.I. (2000). Blockade by pertussis toxin of the opioid effect on guinea pig ileum. Contractility and electrophysiological neuronal recording. *Neurosci. Lett.,* **291,** 131 – 134.

ALLESCHER, H.D., STORR, M., BRECHMANN, C., HAHN, A. & SCHUSDZIARRA, V. (2000). Modulatory effect of endogenous and exogenous opioids on the excitatory reflex pathway of the rat ileum. *Neuropeptides,* **34,** 62 – 68.

BAUER, A.J., SARR, M.G. & SZURSZEWSKI, J.H. (1991). Opioids inhibit neuromuscular transmission in circular muscle of human and baboon jejunum. *Gastroenterology,* **101,** 970 – 976.

BERZETEI-GURSKE, I.P. & TOLL, L. (1992). The mu-opioid activity of kappa-opioid receptor agonist compounds in the guinea pig ileum. *Eur. J. Pharmacol.,* **212,** 283 – 286.

BILSKY, E.J., CALDERON, S.N., WANG, T., BERNSTEIN, R.N., DAVIS, P., HRUBY, V.J., MCNUTT, R.W., ROTHMAN, R.B., RICE, K.C. & PORRECA, F. (1995). SNC 80, a selective, nonpeptidic and systemically active opioid delta agonist. *J. Pharmacol. Exp. Ther.,* **273,** 359 – 366.

BITAR, K.N. & MAKHLOUF, G.M. (1985). Selective presence of opiate receptors on intestinal circular muscle cells. *Life Sci.,* **37,** 1545 – 1550.

BROWN, D.R., POONYACHOTI, S., OSINSKI, M.A., KOWALSKI, T.R., PAMPUSCH, M.S., ELDE, R.P. & MURTAUGH, M.P. (1998). Delta-opioid receptor mRNA expression and immunohistochemical localization in porcine ileum. *Dig. Dis. Sci.,* **43,** 1402 – 1410.

CHAMOUARD, P., KLEIN, A., MARTIN, E., ADLOFF, M. & ANGEL, F. (1993). Regulatory role of enteric kappa opioid receptors in human colonic motility. *Life Sci.,* **53,** 1149 – 1156.

CHERUBINI, E., MORITA, K. & NORTH, R.A. (1985). Opioid inhibition of synaptic transmission in the guinea-pig myenteric plexus. *Br. J. Pharmacol.,* **85,** 805 – 817.

CHERUBINI, E. & NORTH, R.A. (1985). Mu and kappa opioids inhibit transmitter release by different mechanisms. *Proc. Natl. Acad. Sci. U.S.A.,* **82,** 1860 – 1863.

CORBETT, A.D., GILLAN, M.G.C., KOSTERLITZ, H.W., MCKNIGHT, A.T., PATERSON, S.J. & ROBSON, L.E. (1984). Selectivities of opioid peptide analogues as agonists and antagonists at the delta-receptor. *Br. J. Pharmacol.,* **83,** 271 – 279.

COSENTINO, M., MARINO, F., BOMBELLI, R., FERRARI, M., RASINI, E., GIARONI, C., LECCHINI, S. & FRIGO, G. (1997). Modulation of neurotransmitter release by opioid mu- and kappa-receptors from adrenergic terminals in the myenteric plexus of the guinea-pig colon: effect of alpha2-autoreceptor blockade. *Neurosci. Lett.,* **222,** 75 – 78.

COSENTINO, M., MARINO, F., DEPONTI, F., GIARONI, C., SOMAINI, L., LEONI, O., LECCHINI, S. & FRIGO, G. (1995). Tonic modulation of neurotransmitter release in the guinea-pig myenteric plexus: effect of mu and kappa opioid receptor blockade and of chronic sympathetic denervation. *Neurosci. Lett.,* **194,** 185 – 188.

COSTA, M., BROOKES, S.J.H., STEELE, P.A., GIBBINS, I., BURCHER, E. & KANDIAH, C.J. (1996). Neurochemical classification of myenteric neurons in the guinea-pig ileum. *Neuroscience,* **75,** 949 – 967.

COUPAR, I.M. (1995). The peristaltic reflex in the rat ileum: evidence for functional mu- and delta-opiate receptors. *J. Pharm. Pharmacol.,* **47,** 643 – 646.

CULPEPPER-MORGAN, J.A., KREEK, M.J., HOLT, P.R., LAROCHE, D., ZHANG, J. & O'BRYAN, L. (1988). Orally administered kappa as well as mu opiate agonists delay gastrointestinal transit time in the guinea pig. *Life Sci.,* **42,** 2073 – 2077.

DE LUCA, A. & COUPAR, I.M. (1996). Insights into opioid action in the intestinal tract. *Pharmacol. Ther.,* **69,** 103 – 115.

DHAWAN, B.N., CESSELIN, F., RAGHUBIR, R., REISINE, T., BRADLEY, P.B., PORTOGHESE, P.S. & HAMON, M. (1996). International Union of Pharmacology. XII. Classification of opioid receptors. *Pharmacol. Rev.,* **48,** 567 – 592.

FURNESS, J.B. (2000). Types of neurons in the enteric nervous system. *J. Auton. Nerv. Syst.,* **81,** 87 – 96.

GIULIANI, S., LECCI, A., TRAMONTANA, M. & MAGGI, C.A. (1996). Role of kappa opioid receptors in modulating cholinergic twitches in the circular muscle of guinea-pig colon. *Br. J. Pharmacol.,* **119,** 985 – 989.

GOMES, I., JORDAN, B.A., GUPTA, A., TRAPAIDZE, N., NAGY, V. & DEVI, L.A. (2000). Heterodimerization of mu and delta opioid receptors: a role in opiate synergy. *J. Neurosci.,* **20,** 1 – 5.

HANDA, B.K., LAND, A.C., LORD, J.A., MORGAN, B.A., RANCE, M.J. & SMITH, C.F. (1981). Analogues of beta-LPH61-64 possessing selective agonist activity at mu-opiate receptors. *Eur. J. Pharmacol.,* **70,** 531 – 540.

HEINEMANN, A., SHAHBAZIAN, A., BARTHO, L. & HOLZER, P. (1999). Different receptors mediating the inhibitory action of exogenous ATP and endogenously released purines on guinea-pig intestinal peristalsis. *Br. J. Pharmacol.,* **128,** 313 – 320.

HIRNING, L.D., PORRECA, F. & BURKS, T.F. (1985). Mu, but not kappa, opioid agonists induce contractions of the canine small intestine ex vivo. *Eur. J. Pharmacol.,* **109,** 49 – 54.

HOLZER, P., LIPPE, I.T., HEINEMANN, A. & BARTHO, L. (1998). Tachykinin NK_1 and NK_2 receptor-mediated control of peristaltic propulsion in the guinea-pig small intestine in vitro. *Neuropharmacology,* **37,** 131 – 138.

JENKINSON, D.H. (1991). How we describe competitive antagonists: three questions of usage. *Trends Pharmacol. Sci.,* **12,** 53 – 56.

JOHNSON, S.M., COSTA, M. & HUMPHREYS, C.M.S. (1988). Opioid mu and kappa receptors on axons of cholinergic excitatory motor neurons supplying the circular muscle of guinea-pig ileum. *Naunyn Schmiedeberg's Arch. Pharmacol.,* **338,** 397 – 400.

JOHNSON, S.M., COSTA, M., HUMPHREYS, C.M.S. & SHEARMAN, R. (1987). Inhibitory effects of opioids in a circular muscle-myenteric plexus preparation of guinea-pig ileum. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **336,** 419 – 424.

KOJIMA, Y., TAKAHASHI, T., FUJINA, M. & OWYANG, C. (1994). Inhibition of cholinergic transmission by opiates in ileal myenteric plexus is mediated by kappa receptor. Involvement of regulatory inhibitory G protein and calcium N-channels. *J. Pharmacol. Exp. Ther.,* **268,** 965 – 970.

KROMER, W. (1993). Gastrointestinal effects of opioids. In *Opioids*. ed. Herz, A. Handbook of Experimental Pharmacology, vol. 104/ II, pp. 163 – 190. Berlin: Springer.

KROMER, W. & SCHMIDT, H. (1982). Opioids modulate intestinal peristalsis at a site of action additional to that modulating acetylcholine release. *J. Pharmacol. Exp. Ther.,* **223,** 271 – 274.

LENARD, L., HALMAI, V. & BARTHO, L. (1999). Morphine contracts the guinea pig ileal circular muscle by interfering with a nitric oxide mediated tonic inhibition. *Digestion,* **60,** 562 – 566.

MARKI, A., MONORY, K., ÖTVÖS, F., TOTH, G., KRASSNIG, R., SCHMIDHAMMER, H., TRAYNOR, J.R., ROQUES, B.P., MALDONADO, R. & BORSODI, A. (1999). Mu-opioid receptor specific antagonist cyprodime: characterization by in vitro radioligand and $-^{35}S-GTP\gamma S$ binding assays. *Eur. J. Pharmacol.,* **383,** 209 – 214.

PATACCHINI, R., BARTHO, L. & MAGGI, C.A. (1997). Characterization of receptors mediating contraction induced by tachykinins in the guinea-pig isolated common bile duct. *Br. J. Pharmacol.,* **122,** 1633 – 1638.

POONYACHOTI, S., PORTOGHESE, P.S. & BROWN, D.R. (2001). Characterization of opioid receptors modulating neurogenic contractions of circular muscle from porcine ileum and evidence that delta- and kappa-opioid receptors are coexpressed in myenteric neurons. *J. Pharmacol. Exp. Ther.,* **297,** 69 – 77.

PORTOGHESE, P.S., SULTANA, M. & TAKEMORI, A.E. (1988). Naltrindole, a highly selective and potent non-peptide delta opioid receptor antagonist. *Eur. J. Pharmacol.,* **146,** 185 – 186.

SCHMIDHAMMER, H., BURKARD, W.P., EGGSTEIN-AEPPLI, L. & SMITH, C.F. (1989). Synthesis and biological evaluation of 14-alkoxymorphinans. 2. (-)-N-(cyclopropylmethyl)-4,14-dimethoxymorphinan-6-one, a selective mu opioid receptor antagonist. *J. Med. Chem.,* **32,** 418 – 421.

SCHMIDHAMMER, H., SCHRATZ, A. & MITTERDORFER, J. (1990). 14-methoxymetopon, an extremely potent opioid agonist. *Helv. Chim. Acta,* **73,** 1784 – 1787.

SHAHBAZIAN, A., SCHULIGOI, R., HEINEMANN, A., PESKAR, B.A. & HOLZER, P. (2001). Disturbance of peristalsis in the guinea-pig isolated small intestine by indomethacin, but not cyclo-oxygenase isoform-selective inhibitors. *Br. J. Pharmacol.,* **132,** 1299 – 1309.

SHAW, J.S., CARROLL, J.A., ALCOCK, P. & MAIN, B.G. (1989). ICI 204448: a kappa-opioid agonist with limited access to the CNS. *Br. J. Pharmacol.,* **96,** 986 – 992.

SPETEA, M., ERLANDSSON HARRIS, H., BERZETEI-GURSKE, I.P., KLARESKOG, L. & SCHMIDHAMMER, H. (2001). Binding, pharmacological and immunological profiles of the delta-selective opioid receptor antagonist HS 378. *Life Sci.,* **69,** 1775 – 1782.

STERNINI, C., SPANN, M., ANTON, B., KEITH, D.E., BUNNETT, N.W., VON ZASTROW, M., EVANS, C. & BRECHA, N.C. (1996). Agonist-selective endocytosis of mu opioid receptor by neurons in vivo. *Proc. Natl. Acad. Sci. U.S.A.,* **93,** 9241 – 9246.

TAGUCHI, A., SHARMA, N., SALEEM, R.M., SESSLER, D.I., CARPENTER, R.L., SEYEDSADR, M. & KURZ, A. (2001). Selective postoperative inhibition of gastrointestinal opioid receptors. *New Engl. J. Med.,* **345,** 935 – 940.

TAKEMORI, A.E., HO, B.Y., NAESETH, J.S. & PORTOGHESE, P.S. (1988). Nor-binaltorphimine, a highly selective kappa-opioid antagonist in analgesic and receptor binding assays. *J. Pharmacol. Exp. Ther.,* **246,** 255 – 258.

TAVANI, A., GAMBINO, M.C. & PETRILLO, P. (1984). The opioid kappa-selective compound U-50,488H does not inhibit intestinal propulsion in rats. *J. Pharm. Pharmacol.,* **36,** 343 – 344.

TELFORD, G.L., CAUDILL, A., CONDON, R.E. & SZURSZEWSKI, J.H. (1988). Ketocyclazocine, a kappa-opioid receptor agonist, and control of intestinal myoelectric activity in dogs. *Am. J. Physiol.,* **255,** G566 – G570.

TONINI, M., WATERMAN, S.A., CANDURA, S.M., COCCINI, T. & COSTA, M. (1992). Sites of action of morphine on the ascending excitatory reflex in the guinea-pig small intestine. *Neurosci. Lett.,* **144,** 195 – 198.

TRENDELENBURG, P. (1917). Physiologische und pharmakologische Versuche über die Dünndarmperistaltik. *Arch. exp. pathol. Pharmakol.,* **81,** 55 – 129.

VECCHIETTI, V., GIORDANI, A., GIARDINA, G., COLLE, R. & CLARKE, G.D. (1991). (2S)-1-(arylacetyl)-2-(aminomethyl)piperidine derivatives: novel, highly selective kappa opioid analgesics. *J. Med. Chem.,* **34,** 397 – 403.

WATERMAN, S.A., COSTA, M. & TONINI, M. (1992). Modulation of peristalsis in the guinea-pig isolated small intestine by exogenous and endogenous opioids. *Br. J. Pharmacol.,* **106,** 1004 – 1010.

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