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Characterisation of opioid receptors involved in modulating circular and longitudinal muscle contraction in the rat ileum

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1 The aim of the present investigation was to characterise the opioid receptor subtypes present in the rat ileum using a method that detects drug action on the enteric nerves innervating the circular and longitudinal muscles.

2 Neurogenic contractions were reversibly inhibited by morphine (circular muscle pEC_{50}, 6.43 ± 0.17, E_{max} $81.7 \pm 5.0\%$; longitudinal muscle pEC_{50}, 6.65 ± 0.27, E_{max} $59.7 \pm 7.8\%$), the μ-opioid receptor-selective agonist, DAMGO ([D-Ala^2,*N*-Me-Phe^4,Gly^5-ol]enkephalin acetate) (circular pEC_{50}, 7.85 ± 0.04, E_{max} $97.8 \pm 3.6\%$; longitudinal pEC_{50}, 7.35 ± 0.09, E_{max} $56.0 \pm 6.1\%$), the δ-selective agonist DADLE ([D-Ala^2,D-Leu^5]enkephalin acetate) (circular pEC_{50}, 7.41 ± 0.17, E_{max}, $93.3 \pm 8.4\%$; longitudinal pEC_{50}, 6.31 ± 0.07, E_{max} $66.5 \pm 5.2\%$) and the κ-selective agonist U 50488H (*trans*-($\pm$)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide methanesulphonate) (circular pEC_{50}, 5.91 ± 0.41, E_{max}, $83.5 \pm 26.8\%$; longitudinal pEC_{50}, 5.60 ± 0.08, E_{max} $74.3 \pm 7.2\%$). Agonist potencies were generally within expected ranges for activity at the subtype for which they are selective, except for U 50488H, which was less potent than expected.

3 The μ and δ receptor-selective antagonists, CTAP (H-D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH_2) and naltrindole, caused progressive, parallel rightward shifts in the DAMGO and DADLE curves, respectively. Analysis indicated conformity to theoretical simple competitive antagonist behaviour. U 50488H effects were insensitive to the κ-selective antagonist, n-BNI. A high concentration (1 μM) of naltrexone caused apparent potentiation of U 50488H effects.

4 CTAP pK_B estimates were consistent with previously reported values for μ receptor antagonism (circular 7.84 ± 0.17, longitudinal 7.64 ± 0.35). However, the naltrindole pK_B estimates indicated lower antagonist potency than expected (circular 8.22 ± 0.23, longitudinal 8.53 ± 0.35).

5 It is concluded that μ and possibly atypical δ receptors (but not κ receptors) mediate inhibition of contraction in this model. Nonopioid actions of U 50488H are probably responsible for the inhibitory effects seen with this compound.

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Abbreviations: CTAP, H-D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH_2 (disulphide bridge 2–7); DADLE, [D-Ala^2,D-Leu^5]enkephalin acetate; DAMGO, [D-Ala^2,*N*-Me-Phe^4,Gly^5-ol]enkephalin acetate; n-BNI, *nor*-binaltorphimine dihydrochloride; U 50488H, *trans*-($\pm$)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide methanesulphonate; U 69593, (+)-(5α,7α,8β)-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro[4.5]dec-8-yl]-benzeneacetamide

Introduction

The inhibitory effects of opioid drugs on gastrointestinal transit in the rat have been the subject of extensive research. However, only a limited number of reports have been made regarding the muscle contractility changes that give rise to these effects. In particular, effects on circular muscle have received very little attention. Circular muscle contractility plays a dominant role in segmentation and peristaltic propulsion in the gut (Kosterlitz & Lees, 1964), and its sensitivity to many drugs has been shown to differ from that of longitudinal muscle contractility (Brownlee & Harry, 1963; Coupar, 1999).

The antitransit effects of opioid drugs arise from changes in both motility and secretion resulting from their activation of opioid receptors located in the gut wall (Bianchi *et al*., 1982; 1983). Immunohistochemical studies have revealed that the opioid receptor subtypes, μ, δ and κ, are present in neural tissue of the rat enteric nervous system (ENS), but not in smooth muscle cells (Sternini *et al*., 1995; Bagnol *et al*., 1997; Gray *et al*., unpublished observations). Although transit arrest is a common effect of opioids in mammals, underlying secretomotor changes appear to vary between species (Miller & Hirning, 1989). In the rat, the issue has been complicated by reports of differing tissue responses under different experimental conditions (Burks, 1976; Coupar & De Luca, 1994); studies have examined drug effects on unstimulated tissues, as well as tissues stimulated exogenously with electrical pulses.

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Offsetting this, efforts to study opioid and opioid receptor pharmacology have capitalised on the availability of highly subtype-selective agonists and antagonists.

With respect to transit in rats, *in vivo* studies employing the charcoal meal method have indicated that μ and δ receptor activation causes transit slowing, but κ receptor activation has little or no effect (Tavani *et al*., 1984; 1990; La Regina *et al*., 1988). With regard to contractility, an *in vivo* study using anaesthetised rats indicated that morphine caused transient tonic and phasic increases in small intestine intraluminal pressure, which was presumably due to circular and/or longitudinal muscle contraction (Burks, 1976). In apparent contrast, an *in vitro* study indicated that both μ and δ receptor activation had an inhibitory influence on the peristaltic reflex of the rat ileum (Coupar, 1995). *In vitro* studies using electrical stimulation have similarly identified an inhibitory influence of δ receptors (but not of μ receptors), on longitudinal muscle contractions in the rat jejunum (Coupar & De Luca, 1994; Hancock & Coupar, 1994).

The primary aim of this study was to examine the effects mediated by μ, δ and κ opioid receptor subtypes on electrically stimulated neurogenic circular muscle contractions in the rat ileum. The *in vitro* method used in this study also allows for simultaneous measurement of drug effects on the longitudinal muscle (Coupar & Liu, 1996). Therefore, we have taken this opportunity to reinvestigate whether functional μ or κ opioid receptors influence longitudinal muscle contractility, using agonists and antagonists with greater subtype-selectivity than have been used previously. A basic account of these findings has been published as a conference abstract (Gray *et al*., 2004).

Methods

Animals

Tissues were obtained from male and female Hooded Wistar rats (200–500 g) and adult male tricolour guinea pigs (approx. 400 g). The animals were maintained under a 12-h light/dark cycle, and allowed access to standard chow and water *ad libitum* prior to use. Ethical approval was obtained from the Monash University Victorian College of Pharmacy Animal Experimentation Ethics Committee.

Measurement of smooth muscle contractility

The organ bath method used was described in Coupar & Liu (1996), and enables simultaneous measurement of circular and longitudinal muscle contractions. Rats were stunned with a blow to the head and killed by exsanguination. Up to six 8 cm sections of ileum (minimum 10 cm oral to the caecum) were excised and rinsed with 10 ml Krebs -Henseleit solution, containing (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. Each segment was sheathed over and tied to a tissue hook assembly, securely occluding the lower luminal opening. At the unattached end of the tissue segment, a cannula made from polyethylene tubing, filled with Krebs–Henseleit solution, was inserted and tied in position. The tissue/cannula lumen communicated with a pressure transducer (Gould P23ID), producing a closed-lumen set-up. The assembly was then immersed in Krebs–Henseleit solution bubbled with 95% O_2–5% CO_2 in a 30 ml organ bath and maintained at 37°C. The cannula was connected to an isometric force transducer (Grass FT03C) with a thread of cotton, and the tissue tensioned to $1 \times g$. After 30-min equilibration, the lumen was filled with 0.15 ml Krebs–Henseleit solution, and the tissue allowed a further 10-min equilibration. The tissue hook included platinum electrodes for transmural field stimulation, through which pulse trains were delivered. A Grass S48 stimulator supplied (supra-maximal) 40 V square wave pulses of 1 ms duration at a rate of 10 Hz, in 8 s trains to induce neurogenic circular and longitudinal muscle contractions. Pulse trains were applied at intervals of 3 min. Longitudinal and circular muscle contractions were measured by the isometric and pressure transducers, respectively. These dual responses were plotted using a Grass model 79D polygraph.

Complete cumulative concentration–response curves were established to the μ-, δ- and κ-opioid receptor-selective agonists, DAMGO ([D-Ala^2,*N*-Me-Phe^4,Gly^5-ol]enkephalin acetate), DADLE ([D-Ala^2,D-Leu^5]enkephalin acetate) and U 50488H (*trans*-(±)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide methanesulphonate), in the presence of various concentrations of their corresponding receptor-selective competitive antagonist, CTAP (H-D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH_2), naltrindole or n-BNI (*nor*-binaltorphimine dihydrochloride). The observed lack of effect of n-BNI on the U 50488H concentration–response curve prompted the use of the broad-spectrum opioid receptor antagonist, naltrexone, with this agonist. A concentration–response curve was also established to the prototype opioid compound, morphine. Antagonists were added to the bath 10–20 min prior to the first agonist addition, and agonists were added 90 s prior to the stimulation train at which the response was measured. At least three different antagonist concentrations were used to establish the linearity of antagonist effects, in addition to a control (no antagonist) curve. At least four replicate responses were developed for each antagonist concentration. At the end of the concentration–response determination, stimulation after bath washout verified reversibility of agonist effects and confirmed tissue viability. Rat tissues were discarded after a single concentration–response experiment.

Since experiments indicated low U 50488H potency values and a lack of n-BNI antagonist activity, the chemical integrity of our stocks of these κ ligands was verified by bioassay, using the method of Birch *et al*. (1987). Segments of guinea-pig ileum approximately 3 cm in length were set up in organ baths containing Krebs–Henseleit solution at 37°C and bubbled with 95% O_2–5% CO_2. Enteric nerves were stimulated with single pulses (1 ms duration at 0.1 Hz, with supramaximal voltage) from platinum transmural electrodes.

Drugs

Morphine hydrochloride, manufactured by Macfarlan Smith Ltd (London, U.K.); DAMGO, DADLE, CTAP, naltrindole and n-BNI (*nor*-binaltorphimine dihydrochloride), purchased from Sigma Chemical Co. (St Louis, MO, U.S.A.); U 50488H, a gift from Upjohn, naltrexone, purchased from DuPont Pharmaceuticals (Wilmington, DE, U.S.A.). All compounds were dissolved in distilled water prior to addition to the organ bath.

Data analysis

Experimental data were analysed to obtain pK_B values using the global nonlinear regression method described by Lew & Angus (1995). Advantages offered by this method over the traditional Schild method have been discussed previously (Lew & Angus, 1995). Agonist effects on contraction were expressed as a percentage of complete inhibition of contraction, with preagonist contraction strengths serving as the baseline level. Sigmoidal curves were fitted to individual concentration-responses by nonlinear regression, using the four-parameter logistic equation to obtain location and slope parameters. The equation is

$$y = a + \frac{b}{1 + 10^{-d(\mathrm{pEC}_{50} + \log[A])}} \quad (1)$$

where A is the agonist concentration, a is the basal value, b is the vertical range, and d is the mid-point slope.

A curve was fitted by nonlinear regression to the pEC_{50} values outputted from Eq. (1) regressions, against the corresponding antagonist concentrations, according to the following equation:

$$\mathrm{pEC}_{50} = -\log([B]^s + 10^{-pK}) - \log c \quad (2)$$

where $[B]$ denotes antagonist concentration, pK, $\log c$ and s are fitting parameters, with s being a measure of the molecularity of the antagonist–receptor interaction. s is directly equivalent to the Schild slope factor (Arunlakshana & Schild, 1959), and simple competitive antagonism theoretically produces an s value of 1. The F test comparison method was used to indicate the acceptability of constraining the s value to 1. $P<0.05$ was taken to be significant. When the constraint was permitted and applied, the pK value was taken to be equivalent to the pK_B.

The obtained pK_B value was then used to represent experimental data in a Clark plot, a graph of agonist $\log \mathrm{EC}_{50}$ vs $\log([B] + K_B)$. Analogous to the Schild plot, the Clark plot indicates the graded effect of the antagonist on agonist potency. By convention, the Clark plot is used to illustrate the deviation of experimental data from theoretical antagonism behaviour. In simple competitive antagonism, data theoretically follow a line with a gradient of 1 (the value of s), offset laterally from the origin by the value of $\log c$. pK_B values are read from a Clark plot at the abscissa where $[B] = 0$.

Additional curves were generated from pooled agonist response data to verify curve parallelism over the tested antagonist concentrations. Again, this was carried out using the F test to compare the goodness-of-fit of constrained and unconstrained regression curves to the experimental data. $P<0.05$ was taken to be significant. Tested constraints to E_{max} and slope parameters were based on best-fit values provided by regressions set to yield common values between data sets. The minimum response parameter was constrained to 0%. These curves were also used to provide EC_{50} agonist potency values and a graphical indication of curve positions and shape. The regression curves were not used in subsequent pK_B calculations, however.

Concentration–response and Clark plot data are shown as mean ± s.e.m. *Prism version 4.00* software (GraphPad Software, San Diego, CA, U.S.A.) was used for all computations and graphics.

Results

Effects of agonists

Tissue responses to stimulation were as described previously (Coupar & Liu, 1996), and all data readings were performed within the established 'stability window' of the tissue preparation.

Circular and longitudinal smooth muscle contractions were inhibited by all agonists tested, that is, morphine, DAMGO, DADLE and U 50488H. Agonist potency and E_{max} values are summarised in Table 1. Representative traces showing the effects of DAMGO on circular and longitudinal muscle contractions are shown in Figure 1. Potency values did not significantly differ between circular and longitudinal muscle layers for DAMGO or DADLE, although the longitudinal muscle potency of U 50488H was significantly lower than in circular muscle. However, the E_{max} values of these agonists were all lower for the longitudinal muscle responses than the circular muscle responses. The order of agonist potencies was consistent between the muscle layers, with DAMGO the most potent, followed by DADLE, morphine and U 50488H. Recovery of contractions was apparent after drug washout for each agonist.

Effects of antagonists

Antagonist results are summarised in Table 1. CTAP and naltrindole produced progressive, parallel rightward shifts of the respective DAMGO and DADLE concentration–response curves in circular and longitudinal muscle (Figure 2a and b; Figure 3a and b). Analysis yielded molecularity values (s) of 1.04 ± 0.25 (circular muscle) and 0.87 ± 0.31 (longitudinal

Table 1 Potencies of subtype-selective opioid receptor agonists as inhibitors of transmural stimulation-induced contractions of the rat ileum circular and longitudinal muscle

	pEC_{50} ($-\log M$)		E_{max} (%)			pK_B	
Agonist	*circ.*	*long.*	*circ.*	*long.*	*Antagonist*	*circ.*	*long.*
Morphine	6.43 ± 0.17	6.65 ± 0.27	81.7 ± 5.0	59.7 ± 7.8	[a]	[a]	[a]
DAMGO	7.85 ± 0.04	7.35 ± 0.09	97.8 ± 3.6	56.0 ± 6.1	CTAP	7.84 ± 0.17	7.64 ± 0.35
DADLE	7.41 ± 0.17	6.31 ± 0.07	93.3 ± 8.4	66.5 ± 5.2	Naltrindole	8.22 ± 0.23	8.53 ± 0.35
U 50488H	5.91 ± 0.41	5.60 ± 0.08	83.5 ± 26.8	74.3 ± 7.2	n-BNI	[b]	[b]
U 50488H					Naltrexone	[b]	[b]

[a]Not tested.
[b]Antagonism not observed.
Affinity values of subtype-selective antagonists are also shown.

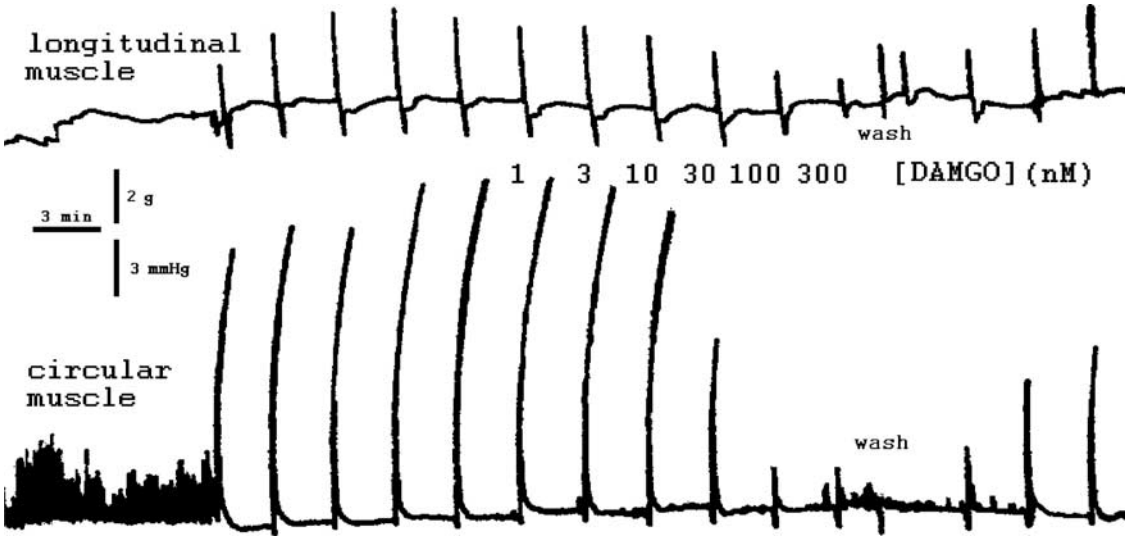

Figure 1 (a) Representative trace of the electrically evoked contractions of the rat ileum, longitudinal muscle (top) and circular muscle (bottom). This example shows the graded inhibition of contractile responses to transmural stimulation (8 s trains of 1 ms pulses at 10 Hz delivered every 3 min) caused by increasing concentrations of DAMGO.

muscle) for CTAP, and 1.10 ± 0.21 (circular muscle) and 0.87 ± 0.31 (longitudinal muscle) for naltrindole. These molecularity values were not significantly different from unity.

Concentrations of n-BNI up to 1 μM failed to alter the concentration–response curve of U 50488H in either circular or longitudinal muscle (Figure 4a and b). At the highest n-BNI concentration tested, a small leftward shift was produced in the circular muscle curve. Bioassay of the U 50488H and n-BNI drug stocks in the guinea-pig ileum longitudinal muscle preparation produced a pEC_{50} of 8.01 ± 0.08 (E_{max}, $84.2 \pm 5.2\%$) for U 50488H and an n-BNI pK_B of 10.3 ± 0.04, values virtually identical to those reported in the literature for activity at κ opioid receptors (Birch *et al*., 1987; Hunter *et al*., 1990). The possibility of agonist activity at μ and δ receptors was then considered. Although the tested n-BNI concentrations had reached levels approaching 100-fold its reported pA_2 at μ and δ receptors (Birch *et al*., 1987), suggesting these receptors were not involved, affirmation was sought with the use of the nonselective opioid receptor antagonist, naltrexone. Naltrexone (1 μM) failed to produce antagonism; in fact, potentiation was observed. Concentration ratios of 0.17 and 0.45 were obtained for circular and longitudinal muscle, respectively.

Discussion

To our knowledge, this is the first *in vitro* study into the effects of opioids on the rat ileum circular muscle. The method used in this study measures the effect of drugs on neurogenic contractile responses of both circular and longitudinal muscle layers (Coupar & Liu, 1996). It has been used previously to show that α_{2D}-adrenoceptors and adenosine A_1 receptors mediate inhibitory effects on neurogenic circular, but not longitudinal muscle contractions in the rat ileum (Liu & Coupar, 1997; Coupar, 1999). The muscular contractions in these studies have appeared to be largely due to neuronal ACh release (as evidenced by sensitivity to atropine), the attenuation of which has been very strongly implicated as a pathway through which the tested agonists caused inhibition of contraction. Similarly, the inhibition of ACh release in opioid-induced inhibition of longitudinal muscle contraction has also been implicated in the stimulated rat small intestine *in vitro* (Hancock & Coupar, 1994). Our present findings show that certain opioid agonists inhibit electrically induced neurogenic circular, as well as longitudinal muscle contractions. The identities of the active sites of these compounds are discussed below.

μ-*Opioid receptor*

The observed potencies of DAMGO are consistent with values reported in standard bioassay preparations for μ-opioid receptors; the guinea-pig ileum (Handa *et al*., 1981; Smith *et al*., 1988) and mouse vas deferens (Handa *et al*., 1981; Smith *et al*., 1988). However, the observed sensitivity of the rat ileum to DAMGO found in our study contrasts with previous findings in the transmurally stimulated longitudinal muscle preparation of the rat jejunum (Coupar & De Luca, 1994; Hancock & Coupar, 1994). The parallel displacement of the DAMGO concentration–effect curve by CTAP and its affinity value derived from this data also indicates μ receptor activity. The CTAP pK_B values calculated from the circular and longitudinal muscle data are consistent with findings in the guinea-pig ileum (Kramer *et al*., 1989).

δ-*Opioid receptor*

Literature pEC_{50} values for DADLE cover the range 7.3-9.4 in the standard functional assays of δ receptors. The present values fall towards the lower end of this range, and are very similar to the hamster vas deferens value of 7.3 (Miller & Shaw, 1985). In addition, the values overlie the reported potency in rat jejunum, an intestinal region anatomically contiguous with the ileum, and in which functional δ receptors have been well established (Hancock & Coupar, 1994). Greater

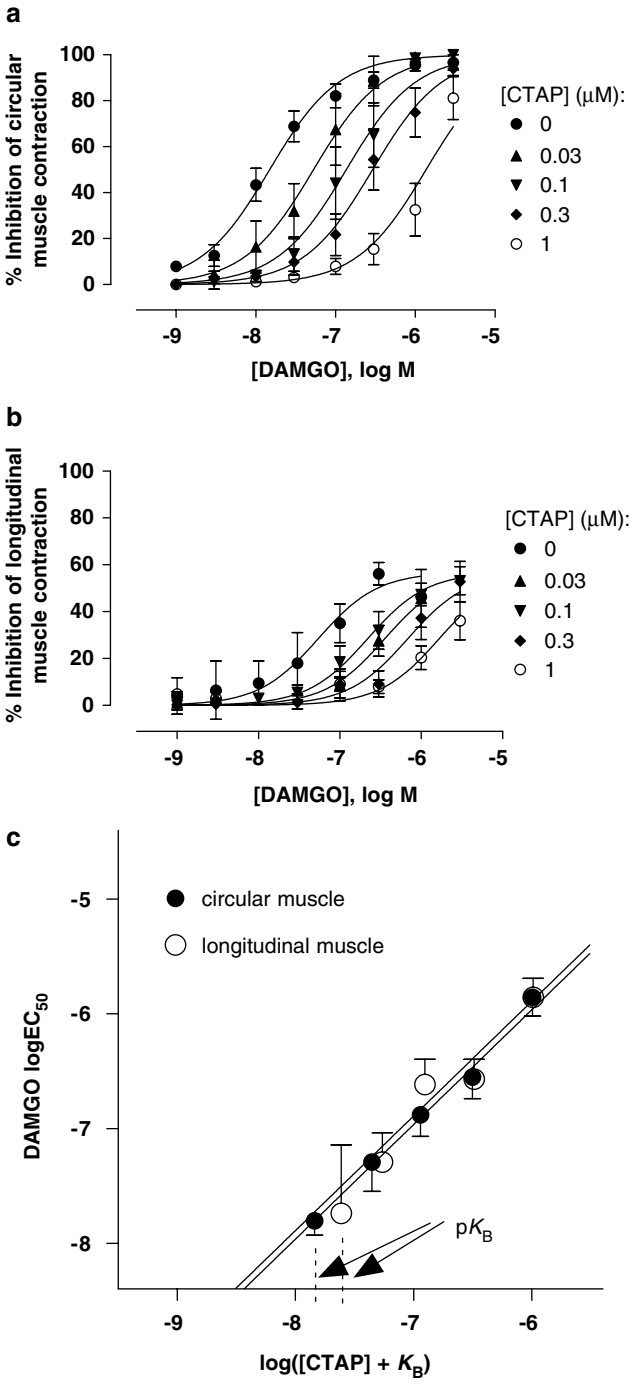

Figure 2 The concentration–response relationship of DAMGO, and DAMGO in the presence of various concentrations of CTAP in circular (a) and longitudinal (b) smooth muscle of the rat ileum. Nonlinear curve fits to pooled data are shown, with common height and slope constraints. The Clark plot (c) indicates the effect of increasing antagonist (CTAP) concentrations on the potency ($\log EC_{50}$) of DAMGO in the different muscle layers. Error bars represent the mean ± s.e.m. of four to 12 determinations per point. The lines are plots of DAMGO $\log EC_{50} = \log([CTAP] + K_B) + \log c$, for circular and longitudinal muscle, representing the 'theoretical lines' for simple competitive antagonism. The proximity of the lines reflects the similarity of the differences between the antagonist pK_B and agonist control curve pEC_{50}, for the different muscle layers.

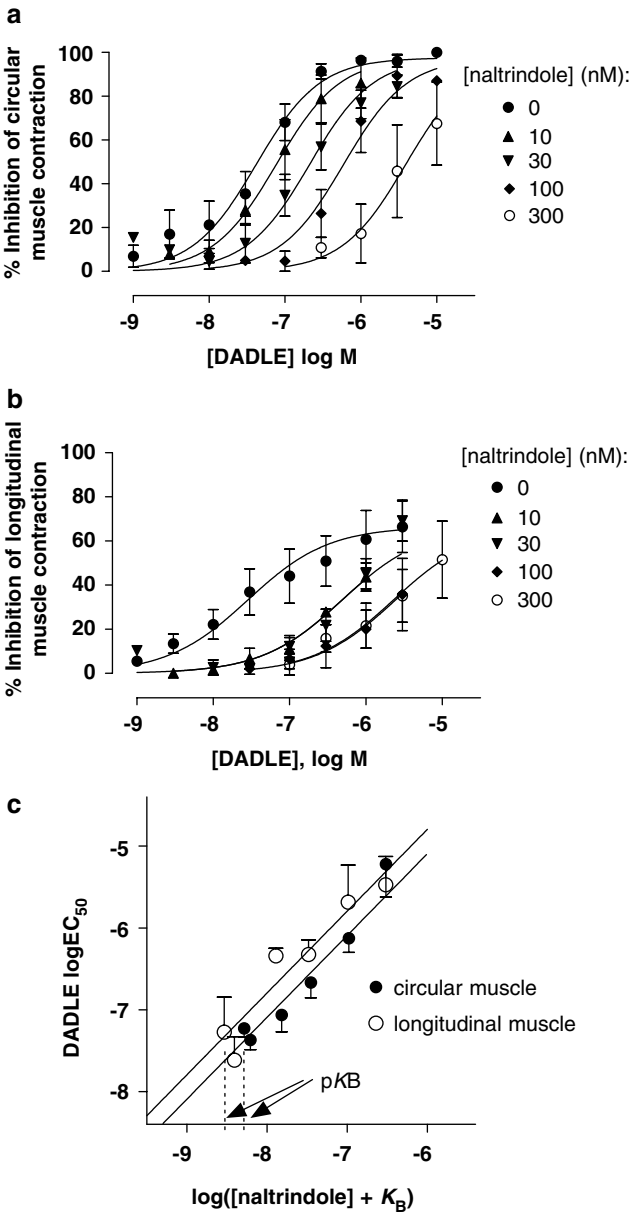

Figure 3 The concentration–response relationship of DADLE, and DADLE in the presence of various concentrations of naltrindole in circular (a) and longitudinal (b) smooth muscle of the rat ileum. Nonlinear curve fits to pooled data are shown, with common height and slope constraints. The Clark plot (c) indicates the effect of increasing antagonist (CTAP) concentrations on the potency ($\log EC_{50}$) of DADLE in the different muscle layers. Error bars represent the mean ± s.e.m. of four to 12 determinations per point. The lines are plots of DADLE $\log EC_{50} = \log([naltrindole] + K_B) + \log c$, for circular and longitudinal muscle, representing the 'theoretical lines' for simple competitive antagonism. The proximity of the lines reflects the similarity of the differences between the antagonist pK_B and agonist control curve pEC_{50}, for the different muscle layers.

DADLE potency has been reported in the guinea-pig ileum (Corbett *et al*., 1984) and mouse vas deferens (Smith *et al*., 1988). The observed naltrindole affinity values (K_B) for circular and longitudinal muscle responses were approximately 10-fold less than what would be predicted from previous reports, in rat jejunum (Hancock & Coupar, 1994) and mouse

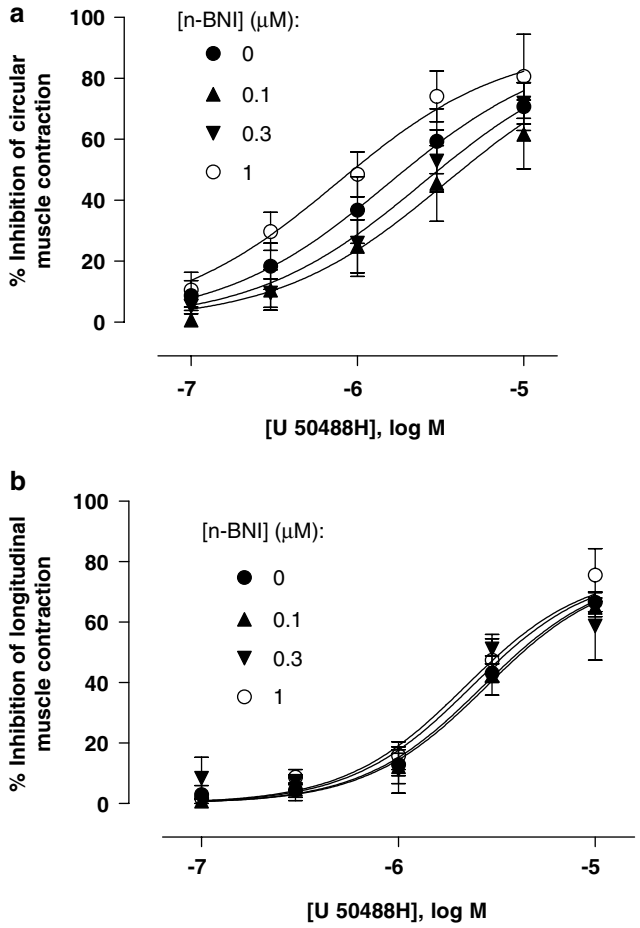

Figure 4 The concentration–response relationship of U 50488H and in the presence of various concentrations of n-BNI in circular (a) and longitudinal (b) smooth muscle of the rat ileum. Nonlinear curve fits to pooled data are shown, with common height and slope constraints. The lack of effect exerted by n-BNI on the control curve suggests a nonopioid mechanism of U 50488H action.

vas deferens (Portoghese *et al*., 1988; Rogers *et al*., 1990). These reports place the pK_B of naltrindole in these δ receptor preparations in the range of 9.6–10.2. The observed affinities were still approximately 10-fold greater than the expected value for antagonism at μ receptors, however (Portoghese *et al*., 1988; Rogers *et al*., 1990). The reasons for this deviation are not clear. Variations in δ antagonist affinity have been reported in brain homogenate assays of adenylyl cyclase activity (Buzas *et al*., 1994; Noble & Cox, 1995). This has been interpreted as being suggestive of δ receptor heterogeneity, although a genetic basis for this conclusion is not currently established. A possible explanation might be modified receptor properties arising from receptor oligomerisation. δ receptor oligomerisation has been demonstrated with μ and κ receptors (Jordan & Devi, 1999; Gomes *et al*., 2000), and is associated with modified ligand binding affinities and efficacy. In the case of ENS tissue, the potential for such oligomerisation appears to exist; cellular co-expression of μ and δ receptors has been shown electrophysiologically in guinea-pig myenteric plexus neurons (Egan & North, 1981). In the rat ileum, double-labelling immunohistochemistry has indicated μ/δ co-expression is widespread in myenteric and submucous plexus neurons (Gray *et al*., unpublished observations).

On the balance of the available evidence, we consider it likely that DADLE acts *via* δ-opioid receptors to inhibit electrically induced neurogenic contractions. These receptors may be atypical in nature, possibly owing to the reasons outlined above.

κ-*Opioid receptor*

The observed inhibitory potencies of U 50488H were 100- to 1000-fold lower than previously reported in the guinea-pig ileum (Birch *et al*., 1987; Hunter *et al*., 1990; Choi *et al*., 1992). This brings into question the role for the κ receptor as an inhibitory influence on neurogenic excitation in the rat ileum. Moreover, U 50488H effects were not sensitive to n-BNI or naltrexone. n-BNI has been consistently reported as possessing subnanomolar antagonist activity at κ receptors, yet concentrations up to 1 μM failed to produce any detectable change in the agonist concentration–response curve in the present experiments. Similarly, the concentration of naltrexone used would be expected to cause large rightwards shifts to opioid receptor-mediated responses (Takemori & Portoghese, 1984). It therefore appears highly unlikely that the observed responses to U 50488H arise from the activation of opioid receptors.

The present *in vitro* results correlate with those obtained *in vivo*. For example, U 50488H did not affect gastrointestinal transit in the rat, even at doses causing profound CNS effects (Tavani *et al*., 1984). A number of other studies have indicated that the role for κ receptors is minor (at best) in opioid-induced constipation in rats (La Regina *et al*., 1988; Tavani *et al*., 1990). Nonetheless, κ receptors were considered a reasonable subject for examination. This is partly because they mediate strong inhibitory effects on contractility and transit in several mammals, including the guinea-pig (Culpepper-Morgan *et al*., 1995) and humans (De Schepper *et al*., 2004). Secondly, κ receptors are located in close proximity to μ and δ receptors in the rat ENS. In addition, the κ-selective agonist U 69593 ((+)-(5α,7α,8β)-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro[4.5]dec-8-yl]-benzeneacetamide) has been shown to antagonise morphine constipation, and cause minor reduction in gastrointestinal transit in rats, with uncertainty surrounding the involvement of κ receptors (La Regina *et al*., 1988).

Nonopioid actions have previously been attributed to U 50488H and related κ opioid arylacetamides in a variety of settings (Alzheimer & ten Bruggencate, 1990; Joshi *et al*., 2000; Su *et al*., 2002). As in the present study, these effects have been characterised by low (μM) potency and insensitivity to n-BNI and nonselective opioid antagonists. The study of these effects has been pursued in a noxious colonic distension model in rats, with observation of a variety of end points (Joshi *et al*., 2000; Su *et al*., 2002). A local-anaesthetic-like blockade of sodium currents in local sensory neurons is considered the likely mechanism, and is supported by electrophysiological findings (Joshi *et al*., 2003).

There is insufficient information to determine the basis for the apparent potentiation of U 50488H by naltrexone. Potentiation of nonopioid U 50488H effects has also been reported with the pharmacologically analogous compound, naloxone, in an electrophysiological study of hippocampal CA3 neurons of the guinea-pig (Alzheimer & ten Bruggencate, 1990).

Morphine

The stimulatory effects of morphine reported previously *in vivo* (Burks, 1976) appear to be at odds with opioid agonist-induced inhibition of contraction observed here. However, critical differences in experimental conditions likely account for this. In particular, the use of electrical stimulation in the present study would have created an altered neuronal state. Indeed, the neurotransmitters at play in stimulated and unstimulated conditions appear to differ. In the *in vivo* study, morphine effects appeared to arise from induction of 5-HT release, whereas in the stimulated tissue, inhibition of ACh release appears to be the major mechanism responsible, at least for longitudinal muscle (for a review, see De Luca & Coupar, 1996). The validity and relevance of the electrically stimulated rat intestine is supported by correlations with drug effects on gastrointestinal transit *in vivo*. Significantly, our finding that μ and δ (but not κ) receptors are involved in attenuating neurogenic contractions correlates with *in vivo* antitransit findings reported previously (Tavani *et al*., 1984; 1990; La Regina *et al*., 1988; Tavani *et al*.).

It is interesting that morphine, considered a full agonist at all opioid receptor subtypes, produced E_{max} values that were exceeded by both DAMGO and DADLE. An explanation for this might be functional self-antagonism *via* activation of κ receptors. Alternatively, contractile effects, as previously reported (Burks, 1976), may have mitigated the drug's inhibitory efficacy on responses to stimulation.

In conclusion, this study provides evidence that neurogenic circular and longitudinal smooth muscle contractions are inhibited by activation of μ opioid receptors and δ opioid receptors (which are possibly of an atypical form) in the ileum of the rat. These effects are probably mediated in the cholinergic neuronal pathway in the ENS. These findings provide a mechanical basis for earlier *in vivo* functional observations, in which apparent activation of these opioid receptor subtypes resulted in arrest of gastrointestinal transit. The unusual properties exhibited by U 50488H in this preparation call for further investigation, and the method used here may provide a convenient tool for the future study of nonopioid properties of arylacetamide κ opioids.

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