

ATP and 5-HT are the principal neurotransmitters in the descending excitatory reflex pathway of the guinea-pig ileum

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Abstract Neurotransmission underlying descending excitatory reflexes evoked by distension was studied in opened segments of guinea-pig ileum and compared with peristalsis in intact segments. The opened segments were distended by inflating a balloon against the serosa at the oral end and changes in muscle length recorded from the anal end. Distension elicited contractions in both circular (CM) and longitudinal (LM) muscle layers. Granisetron, a 5-HT₃ receptor antagonist (10 nmol L⁻¹ to 1 µmol L⁻¹) reduced CM contractions (24% control), without affecting the LM. The P2 receptor antagonist, pyridoxal phosphate-6-azophenyl-2',4'-disulphonic acid (PPADS; 10 µmol L⁻¹), reduced CM contractions to 31% and LM contractions to 39%. Hexamethonium (500 µmol L⁻¹) enhanced LM contractions, but had no effect on CM contractions. Granisetron (1 µmol L⁻¹) had no significant effect on the threshold for peristaltic contractions in a modified Trendelenburg preparation, but decreased the decay time of these contractions by 37%. PPADS (10 µmol L⁻¹) had no significant effect in this preparation. Thus, the descending excitatory pathways to CM and LM can be distinguished pharmacologically; the former depend on 5-HT₃ and P2 ATP receptors, the latter are independent of 5-HT₃ receptors. Nicotinic receptors may have little part in either pathway. These properties differ from conventional peristaltic reflexes, which are effectively abolished by nicotinic blockade.

Keywords enteric reflexes, synaptic transmission.

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INTRODUCTION

Polarized intestinal reflexes were shown by Bayliss & Starling^{1–3} to occur in response to a stationary stimulus. Reflexes occurred in the small intestine of the dog, rabbit and cat and also the large intestine of the dog, and consisted of a contraction oral to the stimulus and a relaxation anal to it. This polarization can also be seen electrophysiologically in the guinea-pig small and large intestine, with a depolarization (excitation) detected in the circular muscle layer oral to a stimulus (known as ascending excitation) and a hyperpolarization (inhibition) detected anal (known as descending inhibition).^{4–6} This relaxation/inhibition and contraction/excitation have been thought to propagate along the intestine with the propulsion of the stimulus eliciting these simple reflexes into the next section of intestine, thus forming the complex propulsive movement known as peristalsis.⁷ Recent contractility experiments in the guinea-pig ileum have, however, shown that a stationary stimulus leads to contractions that propagate in both the oral and anal directions (ascending and descending excitation, respectively) with no preceding relaxation in the anal direction.^{8,9} These observations have led to the suggestion that descending excitation may be an important component of peristalsis.⁸

The enteric nervous system mediates these reflexes.¹⁰ The underlying neural pathways consist of sensory neurones sensitive to stretch,^{11,12} mucosal distortion^{12,13} or luminal chemicals,¹⁴ excitatory and inhibitory motor neurones to the circular and longitudinal muscle,^{15,16} and interneurons that run in both ascending and descending directions.¹⁷ There are many different classes of synapse (e.g. sensory neurone to descending interneurone, descending interneurone to excitatory circular muscle motor neurone) at which distinct combinations of neurotransmitter may be employed. The available transmitters include

acetylcholine (ACh),^{18,19} adenosine triphosphate (ATP)^{20,21} and tachykinins.^{18,19} Investigations into the identity of neurotransmitters employed at particular synapses in the descending neuronal pathways have focused on the inhibitory reflex pathway that underlies the hyperpolarization of the circular muscle. In the guinea-pig ileum, these studies have uncovered roles for tachykinins and for ATP, but not for ACh, leaving a large component of transmission that is unaccounted for.^{19,20,22}

Recently, Spencer *et al.*⁹ began to characterize the transmitters involved in the descending excitatory pathway by investigating transmission between descending interneurons. They found that blockade of nicotinic receptors had little effect by itself and purine receptor blockade alone had no effect. Simultaneous blockade of nicotinic and purinergic receptors had, however, a greater effect than nicotinic receptor blockade alone.

The present study aimed to extend these findings by examining the role of purine, nicotinic and serotonin (5-hydroxytryptamine; 5-HT₃) receptors at all points in the descending excitatory pathway activated by distension in the guinea-pig ileum and to compare this reflex with a standard model of peristalsis.

MATERIALS AND METHODS

Tissue

Guinea-pigs of either sex in the weight range 180–350 g were killed by stunning followed by severing of the carotid arteries and spinal cord, in accordance with the University of Melbourne Animal Experimentation Ethics Committee. The abdominal cavity was opened and a 7-cm segment of ileum was removed 10–30 cm from the ileocaecal junction. This was placed into physiological salt solution (composition in mmol L⁻¹: NaCl 118, NaHCO₃ 25, D-glucose 11, KCl 4.8, CaCl₂ 2.5, MgSO₄ 1.2, NaH₂PO₄ 1.0) at room temperature and bubbled with 95% O₂/5% CO₂.

Flat sheet preparation

The lumen was flushed with physiological salt solution and the segment of ileum cut open along the mesenteric border. It was then pinned flat, mucosa facing up in an organ bath (volume 7 mL, lined with Sylgard, Dow Corning, Midland, MI, USA) with the oral end over a balloon embedded into the base of the bath (Fig. 1a). Pins were inserted around the balloon and at the ends of the preparation; this allowed the tissue to move freely from the anal end of the

stimulation region to the recording region. Responses were evoked by distending the balloon (0.1–0.3 mL) and were recorded from both the circular and longitudinal muscle layers at the anal end (approximately 5 cm and 5.75 cm away, respectively) using isotonic length transducers (Harvard App Ltd., Edenbridge, Kent, UK), attached to a Biopac MP100 (see below). A tension of 0.5 g was imposed on most preparations; tissue from smaller guinea-pigs (< 220 g) was placed under a tension of 0.2 g. Pre-warmed (35–36°) physiological salt solution constantly flowed through the bath at a rate of 7–8 mL min⁻¹ and was bubbled with 95% O₂/5% CO₂.

After a 1-h equilibration period, carbachol (muscarinic receptor agonist, 1 μmol L⁻¹) was superfused into the bath to contract the tissue nearly maximally. Reflex responses obtained throughout the experiment were then expressed as a proportion of this contraction. Following recovery from carbachol, reflexes were evoked by distension and changes in muscle length recorded; reflexes were evoked at 5-min intervals until three contractions of similar amplitude were recorded. Receptor antagonists (see below) were then superfused into the bath and allowed to equilibrate for 15 min, with only one antagonist, or antagonist combination, used per tissue. Three further reflexes were evoked in the presence of the antagonist before it was washed out and recovery of reflexes sought. To determine whether the tissue fatigued during the experiment, carbachol was also added at the end and the amplitude of this contraction compared to the initial application; if the second carbachol response was < 80% of the first, the data were discarded.

Spontaneous contractions of either CM or LM (Fig. 1b,c) were often observed without any sign of coupling to the inactive layer. Such coupling would be seen as a lengthening or apparent relaxation of this layer. The spontaneous contractions were often of comparable amplitude to those evoked by distension (Fig. 1b), indicating that it is unlikely that mechanical interactions contributed significantly to the reflex responses.

Tubular preparation

Preparations used for lumenal pressure recording were set up in a modified Trendelenburg arrangement. A 5-cm length of tissue was collected as above, flushed and placed into a bath (volume 10 mL) containing warmed (35–36 °C) physiological salt solution that flowed at 8 mL min⁻¹ and was bubbled with 95% O₂/5% CO₂. Cannulae were inserted into either end and a pressure transducer (Surgicare model PVB 6003)

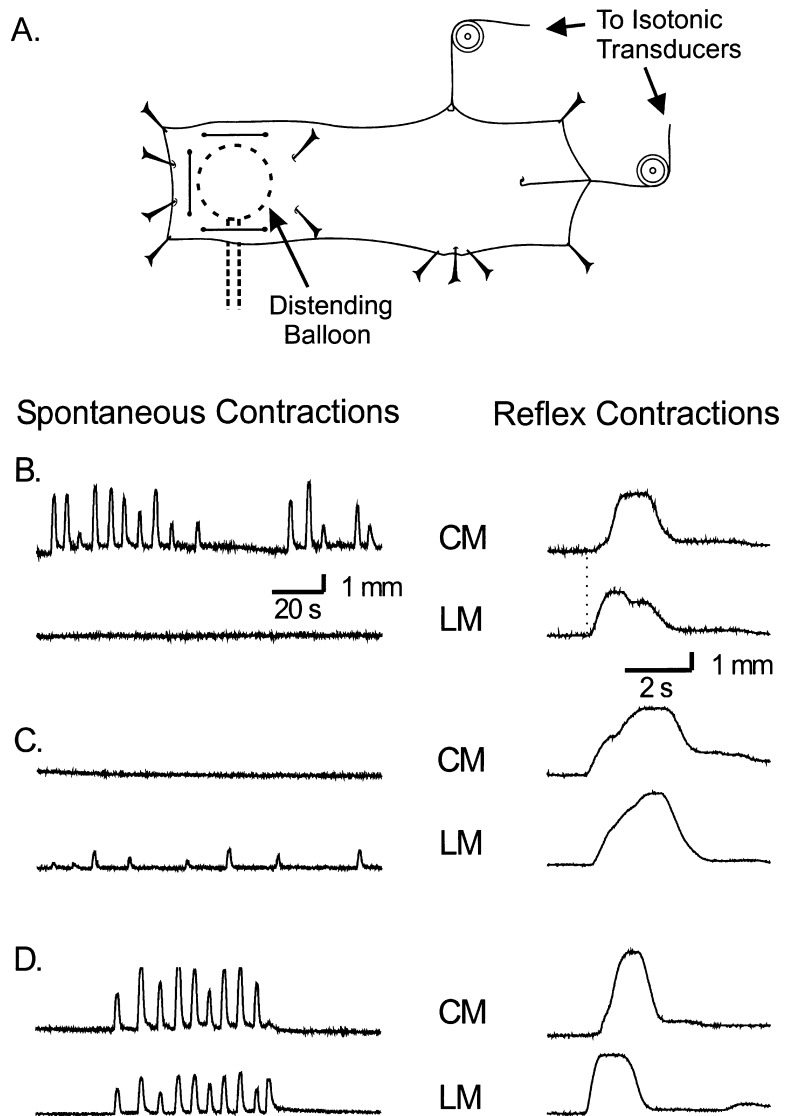


Figure 1 The experimental arrangement and the independent nature of the recordings. (A) A schematic showing the experimental arrangement with the oral end to the left. A balloon embedded in the base of the bath at the oral end (circle) was inflated to elicit a propagating contraction that was recorded as changes in muscle length at the anal end (5–6 cm away) from both the CM and LM. (B,C) Traces showing the independent nature of the contractions as recorded by the isotonic transducers, with spontaneous contractions and reflex contractions (from the same preparation) shown on the right. (D) Traces showing that spontaneous contractions also occur in both muscle layers simultaneously (with reflex contraction from that preparation on the right).

was connected to the anal cannula. The oral cannula was connected to a 50-mL reservoir of physiological salt solution that was covered with a cork and a glass tube in order to maintain a constant pressure. The reservoir was raised 1 cm at a time (corresponding to a 0.76-mmHg increase in pressure) until threshold was reached and peristaltic contractions ensued. The reservoir was then lowered below threshold, which was defined as the pressure at which regular propulsive contractions were initiated. Three consistent bursts of five (or more) contractions were obtained followed by addition of receptor antagonists (see below) to the bath and thus to the serosal surface. These were allowed to equilibrate for at least 10 min before three more bursts of peristaltic contractions were elicited. The antagonist

was then washed out and recovery sought. Once again, only one antagonist was used per tissue.

Data acquisition

Changes in muscle length or pressure were digitized at a sampling rate of 100 s^{-1} using a Biopac MP100 and displayed, analysed and stored on computer using the Acqknowledge 3.2.4 program (both from Biopac Systems Inc, SDR Clinical Technology, Sydney, Australia). For the flat sheet preparations, a sliding potentiometer was attached to the syringe used to inflate the balloon and was driven at 9 V, which provided an accurate record of the onset and duration of the distending stimulus via the MP100.

Drugs

The drugs used in these experiments were prepared as stock solutions in distilled water and included carbachol, hexamethonium, pyridoxal phosphate-6-azophenyl-2',4'-disulphonic acid (PPADS; all from Sigma-Aldrich Fine Chemicals, Sydney, Australia), tetrodotoxin (TTX; Alomone Laboratories, Jerusalem, Israel), granisetron (a kind gift from SmithKline Beecham, Melbourne, Australia) and ondansetron (Glaxo, Greenford, UK). Drugs were dissolved at the final concentration in physiological salt solution before addition to the organ bath.

Analysis of results

Contractions evoked by distension in the flat-sheet preparation were expressed as a proportion of the first carbachol contraction, allowing better comparisons between preparations of different sizes. Responses to distension were measured as contraction amplitude (from baseline to the peak of the contraction). The latency of the response was also measured (from the onset of the stimulus to the onset of the contraction), and along with the distance the contraction propagated, was used to give the propagation rate of the reflex (distance latency⁻¹). In the modified Trendelenburg preparation, the threshold for eliciting peristaltic contractions was determined and the amplitude, rise time and decay time of these contractions were measured for the first five contractions in a burst.

Statistical analyses were done using nonparametric tests, as much of the data was either discontinuous (threshold measurements) or not normally distributed. To analyse differences between control, drug treatment and washout periods, a Friedman nonparametric repeated measures ANOVA was used with Dunn's multiple comparisons test. To determine if there were differences between the effects of different drugs a Kruskal-Wallis nonparametric ANOVA with Dunn's multiple comparisons test was used. For all experiments, $P < 0.05$ was considered significant. All results are shown as mean \pm standard error of the mean (SEM) and, unless otherwise stated, n refers to the number of animals used.

RESULTS

Distension of the oral region of the ileum from the serosal side by inflation of the balloon (0.2–0.3 mL) mounted in the base of the bath elicited contractions in both CM and LM layers; these contractions propagated over the full length of the preparation (5–6 cm). The reflex contraction was 0.29 ± 0.05 of the carbachol

contraction in the CM and 0.36 ± 0.03 in the LM ($n = 55$ from 47 animals). In most preparations, the LM contracted first, the mean latency to onset for all preparations was 1.09 ± 0.08 s (propagation distance 5.75 cm; rate of propagation 5.28 cm s^{-1}) while that in the CM was 1.49 ± 0.08 s (propagation distance 5 cm; rate of propagation 3.36 cm s^{-1} ; $n = 35$ from 30 animals). These contractions were separate and there was no sign of interaction between them in the recording. TTX ($1 \mu\text{mol L}^{-1}$) was added to the bath to confirm that these contractions evoked by distension were neurally mediated and abolished the propagating contractions in both muscle layers, but a small contraction local to the balloon site could still be observed ($n = 6$).

It was found that a distending stimulus of 0.1 mL evoked a small contraction local to the balloon site, but did not evoke a propagating contraction in either muscle layer. The threshold volume required to evoke a propagating contraction was 0.2 mL ($n = 3$). At this volume the mean contraction amplitude was 0.18 ± 0.04 in the CM and 0.18 ± 0.03 in the LM. In these preparations, the amplitude of these contractions in the LM was significantly greater with a 0.3-mL stimulus (0.42 ± 0.02 , $P < 0.05$), but not, however, in the CM (0.25 ± 0.02 , $P > 0.05$). The amplitude of the response was not altered in either muscle layer by varying the duration of the stimulus (CM – 2 s: 0.22 ± 0.05 , 10 s: 0.16 ± 0.04 ; LM – 2 s: 0.4 ± 0.02 , 10 s: 0.45 ± 0.03 ; $P > 0.05$). However, the longer stimulus did sometimes produce a multi-peaked contraction in the LM (not shown).

Effect of 5-HT₃ receptor blockade on the descending contractile reflex

The involvement of 5-HT acting at 5-HT₃ receptors in the neural pathway to either the CM or LM was tested using the specific receptor antagonist granisetron ($1 \mu\text{mol L}^{-1}$, $n = 6$). Addition of granisetron to the bath reduced the amplitude of the contraction in the CM from 0.36 ± 0.1 to 0.09 ± 0.03 ($24 \pm 5\%$ of control, $P < 0.05$) but had no effect on the LM (control 0.34 ± 0.06 ; granisetron 0.30 ± 0.07 ; $P > 0.05$; Figs 2, 3 and 4). The effect of granisetron was concentration dependent with 100 nmol L^{-1} ($n = 6$) and 10 nmol L^{-1} ($n = 6$) concentrations having less of an effect (Fig. 3). Another antagonist specific for 5-HT₃ receptors, ondansetron ($1 \mu\text{mol L}^{-1}$, $n = 6$), had a similar effect to that of granisetron but was less potent. The contraction in the CM was reduced from 0.51 ± 0.13 to 0.32 ± 0.14 ($68 \pm 15\%$ of control; $P < 0.05$; Figs 3 and 4) and there was no effect on the LM (control 0.34 ± 0.06 ; ondansetron 0.29 ± 0.11 ; $P > 0.05$).

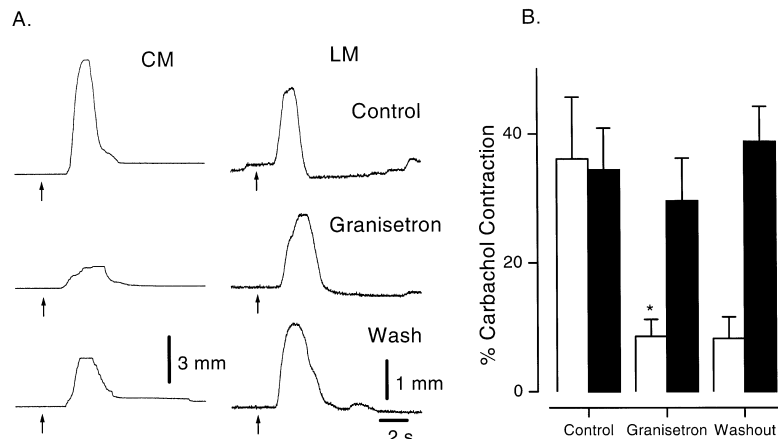


Figure 2 Effect of the 5-HT₃ receptor antagonist granisetron on distension evoked contractions. (A) Muscle length recordings from the anal end of a segment of ileum (5–6 cm long). A balloon was inflated for 2 s at the arrow and a contraction under control conditions (top) was recorded from the circular (CM, left) and the longitudinal (LM, right) muscle. The 5-HT₃ receptor antagonist granisetron (1 μmol L⁻¹) was added to the bath and reflexes again evoked. Granisetron depressed the reflex contraction in the circular muscle but did not affect the longitudinal muscle contraction. This depression was partially reversible following 1 h washout of antagonist. (B) Histogram summarizing data from six animals with the reflex contraction size expressed as a proportion of the carbachol (1 μmol L⁻¹) contraction (see Methods). *Significantly different from control (*P* < 0.05).

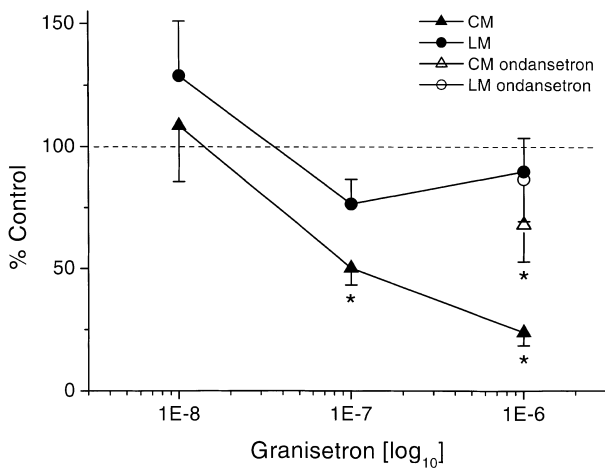


Figure 3 Effect of granisetron and ondansetron on distension evoked contractions. Distension evoked contractile responses of the circular and longitudinal muscle layers expressed as a percentage of the reflex contractions in control (\pm SEM). At a concentration of 10 nmol L⁻¹ (*n* = 6) granisetron had no effect on the contraction amplitude of either muscle layer. At concentrations of 100 nmol L⁻¹ (*n* = 6) and 1 μmol L⁻¹ (*n* = 6) there was a reduction in the circular muscle layer contraction. The effect of granisetron on the circular muscle at 1 μmol L⁻¹ was significantly different to that at 100 nmol L⁻¹ (*P* < 0.05). Ondansetron (1 μmol L⁻¹, *n* = 6) also reduced the contraction amplitude in the circular muscle. *Significantly different from control (*P* < 0.05).

Effect of P2 receptor blockade on the descending contractile reflex

To determine if P2 receptors are involved along the neural pathways to both the CM and LM, the P2 receptor antagonist PPADS (10 μmol L⁻¹, a concentration found by Bian *et al.*²⁰ to be specific for neuronal P2 receptors) was added to the bath (*n* = 6). The distension evoked contraction in the CM was reduced from 0.4 ± 0.18 to 0.15 ± 0.11 (31 ± 11% of control; *P* < 0.05) and in the LM from 0.42 ± 0.07 to 0.19 ± 0.06 (39 ± 10% of control; *P* < 0.05; Figs 4 and 5).

Effect of blocking nicotinic transmission on the descending contractile reflex

The specific nicotinic receptor antagonist hexamethonium (500 μmol L⁻¹) was added to the bath (*n* = 8) and had no effect on the amplitude of the distension evoked contraction in the CM (control 0.27 ± 0.07; hexamethonium: 0.21 ± 0.04; *P* > 0.05). However, in the LM, hexamethonium caused an increase in the amplitude of the response from 0.43 ± 0.09 to 0.55 ± 0.11 (126 ± 10% of control; *P* < 0.05; Fig. 4).

Effects of combining antagonists

To determine if the distension evoked contraction could be further attenuated, or even abolished, by simultaneous blockade of multiple receptor types,

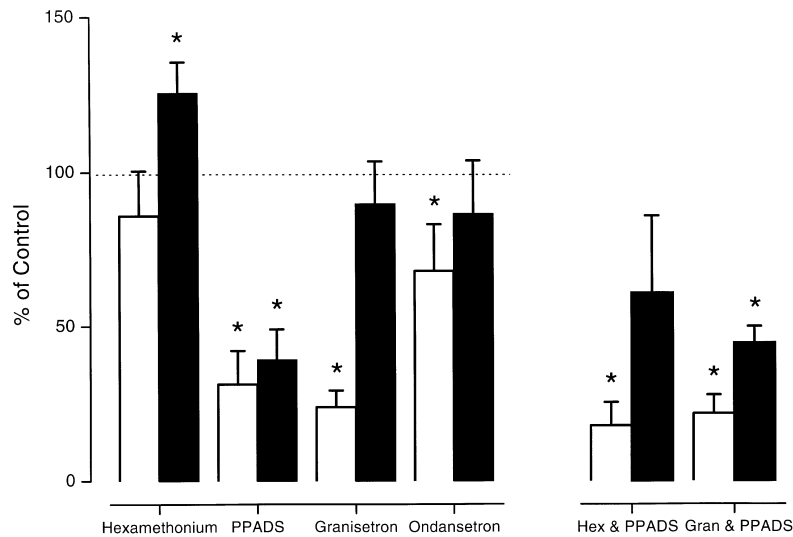


Figure 4 Histogram summarizing the effects of nicotinic, P2 and 5-HT₃ receptor blockade. Values are expressed as a percentage of the control reflex contraction. Blocking nicotinic receptors with hexamethonium ($500 \mu\text{mol L}^{-1}$, $n = 8$) had no effect on the amplitude of the distension evoked contraction in the circular muscle (open bar) but increased that of the longitudinal muscle (filled bar). Blockade of P2 receptors with PPADS ($10 \mu\text{mol L}^{-1}$, $n = 6$) decreased the contraction amplitude in both muscle layers. Blockade of 5-HT₃ receptors with granisetron ($1 \mu\text{mol L}^{-1}$, $n = 6$) or ondansetron ($1 \mu\text{mol L}^{-1}$, $n = 6$) decreased the contraction amplitude of the circular muscle only. Combining hexamethonium ($500 \mu\text{mol L}^{-1}$) and PPADS ($10 \mu\text{mol L}^{-1}$, $n = 6$) had no more effect on the contraction in the circular muscle than PPADS alone. Combining PPADS ($10 \mu\text{mol L}^{-1}$) and granisetron ($1 \mu\text{mol L}^{-1}$, $n = 6$) had no more effect on the circular muscle contraction than either alone. *Significantly different from control ($P < 0.05$).

combinations of antagonists were added to the bath. Hexamethonium ($500 \mu\text{mol L}^{-1}$) and PPADS ($10 \mu\text{mol L}^{-1}$) together ($n = 6$) reduced the amplitude of the CM response from 0.21 ± 0.05 to 0.03 ± 0.02 ($18 \pm 8\%$ of control; $P < 0.05$), but had no significant

effect on the LM (control 0.45 ± 0.12 , hexamethonium/PPADS 0.26 ± 0.06 ; $P > 0.05$; Fig. 4). The effect on both the CM and LM was significantly different from that of hexamethonium ($P < 0.05$) alone, but not PPADS alone ($P > 0.05$).

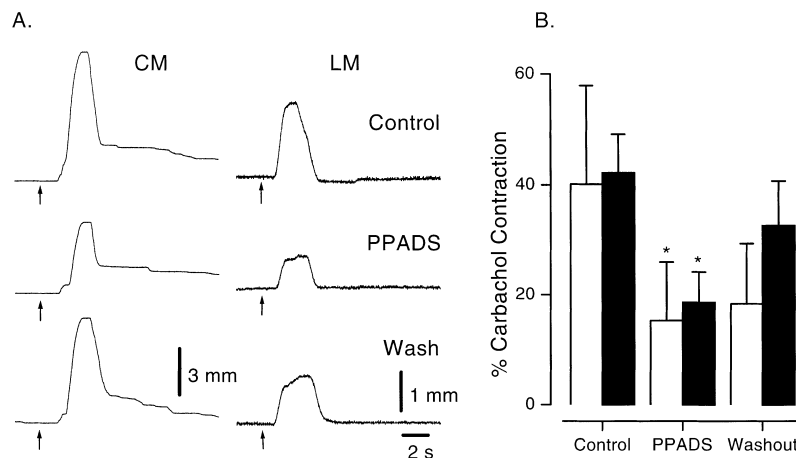


Figure 5 Effect of the P2 receptor antagonist PPADS on distension evoked contractions. (A) Length recordings from the anal end of a segment of ileum. A balloon was inflated for 2 s at the arrow and a contraction under control conditions (top) was recorded from the circular (CM, left) and the longitudinal (LM, right) muscle layers. The P2 receptor antagonist PPADS ($10 \mu\text{mol L}^{-1}$) was added to the bath and reflexes again evoked. PPADS depressed the reflex contraction in both the circular and longitudinal muscle layers. This depression was partially reversible following 1 h washout of antagonist. (B) Histogram summarizing data from six animals with the reflex contraction size expressed as a proportion of the carbachol ($1 \mu\text{mol L}^{-1}$) contraction (see Methods). *Significantly different from control ($P < 0.05$).

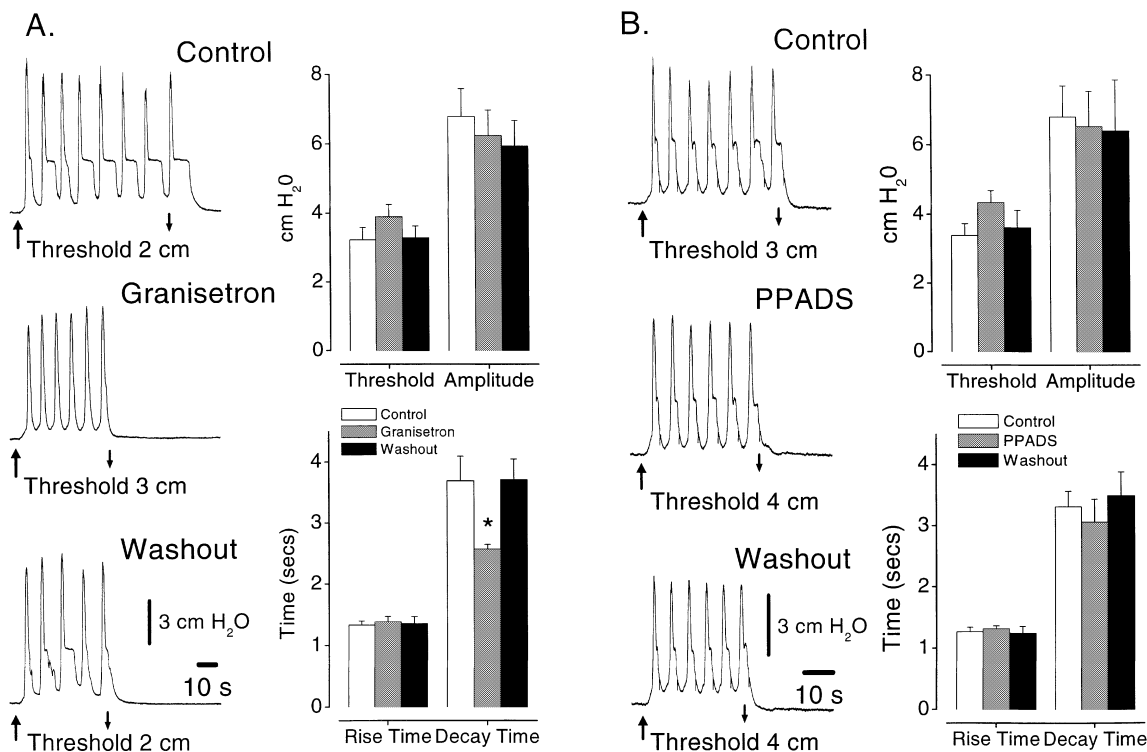


Figure 6 Effect of granisetron and PPADS on peristalsis. (A) Peristaltic contractions were elicited by increasing intraluminal pressure with the increase in pressure occurring at the upward arrow and the lowering of pressure occurring at the downward arrow. Control contractions showed a prominent shoulder during the decay phase of the contraction. This was reduced with the application of granisetron ($1 \mu\text{mol L}^{-1}$, $n = 6$) serosally and partially returned after a 1-h washout period. (B) Addition of PPADS ($10 \mu\text{mol L}^{-1}$, $n = 6$) to the serosal area of the bath had no effect on the peristaltic contractions. *Significantly different from control ($P < 0.05$).

Granisetron ($1 \mu\text{mol L}^{-1}$) and PPADS ($10 \mu\text{mol L}^{-1}$) together ($n = 6$) had a significant effect on both muscle layers with the CM contraction being reduced from 0.23 ± 0.09 to 0.06 ± 0.03 ($22 \pm 6\%$ of control; $P < 0.05$) and the LM contraction from 0.29 ± 0.1 to 0.12 ± 0.04 ($46 \pm 5\%$ of control; $P < 0.05$; Fig. 4). The effect on the CM was not significantly different from that of either PPADS or granisetron alone ($P > 0.05$ in each case). In the LM, this effect was significantly different from that of granisetron alone ($P < 0.05$). The effects of hexamethonium/PPADS and PPADS/granisetron in either muscle layer were not significantly different from each other ($P > 0.05$ in each case).

Effects of 5-HT₃ and P2 antagonists on peristalsis

In the ileum, the descending excitatory reflex seen in the flat sheet appears to be the same as the peristalsis seen in intact preparations (see Discussion). To test this idea, granisetron ($1 \mu\text{mol L}^{-1}$) or PPADS ($10 \mu\text{mol L}^{-1}$) was applied to the serosal (bath) side of a tubular preparation in a modified Trendelenburg

arrangement for pressure recording.²³ Granisetron ($n = 6$, Fig. 6a) increased the pressure threshold to elicit peristaltic contractions from $3.2 \pm 0.4 \text{ cm H}_2\text{O}$ to $3.9 \pm 0.4 \text{ cm H}_2\text{O}$ (123% of control), but this change was not statistically significant ($P > 0.05$). The amplitude of the contractions was unaffected (control $6.8 \pm 0.8 \text{ cm H}_2\text{O}$; granisetron $6.2 \pm 0.7 \text{ cm H}_2\text{O}$; $P > 0.05$), as was the rise time of the contractions (control $1.33 \pm 0.06 \text{ s}$; granisetron $1.38 \pm 0.09 \text{ s}$; $P > 0.05$). However, the decay time of the contractions was decreased from $3.69 \pm 0.4 \text{ s}$ to $2.58 \pm 0.08 \text{ s}$ (73% of control, $P < 0.05$); this effect was reversible upon washout of granisetron. The change in decay time appeared to be associated with the disappearance of a 'hump' or plateau in the relaxation phase of the peristaltic contractions (Fig. 6a).

PPADS ($n = 6$, Fig. 6b) also increased the pressure threshold to elicit peristaltic contractions from $3.4 \pm 0.4 \text{ cm H}_2\text{O}$ to $4.3 \pm 0.4 \text{ cm H}_2\text{O}$ (131% of control), but again this change was not statistically significant ($P > 0.05$). Furthermore, PPADS had no effect on the amplitude of the contractions (control

6.82 ± 0.89 cm H₂O; PPADS 6.53 ± 1.02 cm H₂O; $P > 0.05$), the rise time (control 1.27 ± 0.07 s, PPADS 1.31 ± 0.05 s; $P > 0.05$), or decay time (control 3.31 ± 0.25 s; PPADS 3.06 ± 0.38 s; $P > 0.05$).

DISCUSSION

A primary finding of this study is that the distension-evoked descending contractile responses of the circular and longitudinal muscle layers are regulated via separate neuronal pathways. Synaptic transmission in at least one class of synapse in these pathways is likely to be mediated by P2 receptors, suggesting a major role for ATP as an excitatory neurotransmitter. 5-HT₃ receptors are also involved in the pathway to the circular muscle, but at a different class of synapse. The role of nicotinic receptors, and therefore ACh, appears to be minor. In contrast, neither granisetron nor PPADS had statistically significant effects on peristaltic reflexes in a modified Trendelenburg preparation, with the exception of a change in duration of the propulsive contractions (see below). Peristaltic reflexes are known to be blocked by hexamethonium,²⁴ so taken together these data suggest that the descending excitatory reflex and the peristaltic reflexes are distinct phenomena.

The descending contractile reflex

A transient, stationary distending stimulus elicited an anally propagating contraction in both muscle layers, with the response of the LM generally preceding that of the CM. The contraction in the CM was not preceded by an apparent relaxation indicating that there was no effective mechanical coupling between the two layers and confirming the prior conclusions of Spencer *et al.*,⁸ that a descending relaxation was not prominent under these conditions. In the LM, the contraction was graded above threshold, but in the CM there was an all-or-nothing contraction similar to that recorded during peristalsis.^{25–27} Similarly, the rates of propagation in the LM (5.28 cm s⁻¹) and in the CM (3.36 cm s⁻¹) are similar to the rate of propagation of the propulsive contractions during peristalsis (2.96 ± 0.15 cm s⁻¹), suggesting that the descending excitatory reflex may be the equivalent of peristalsis. Peristalsis has been observed in flat preparations,²⁵ however, there were some apparent differences in that Brookes *et al.*²⁵ found that the LM showed no threshold for contraction. In addition, they found that, above a threshold, the amplitude of the peristaltic contraction was graded. However, Brookes *et al.*²⁵ applied a stretch along the entire length of the segment (similar to an increase in pressure in a tubular preparation: see

below) that increased at a constant rate for approximately 30 s. In the present study, a rapid stretch was applied to the oral end that lasted for no longer than 3 s. These differences in the duration of the stimulus and in the regions being stimulated could account for different contractile behaviour. Thus, further data, discussed below, are needed to allow a definitive conclusion regarding the relationship between the descending excitatory reflex and peristalsis.

Synaptic transmission in the descending excitatory pathway

The application of receptor antagonists to the whole bath enabled a quick evaluation of the receptors (and by inference, the neurotransmitters) involved in synaptic transmission at the many different classes of synapses in the descending excitatory neuronal pathways to both muscle layers.

A novel finding of this study was that 5-HT₃ receptors, and therefore 5-HT, play a large role in excitatory transmission in the pathway to the circular muscle, but not to the longitudinal muscle. Previous studies in the descending inhibitory pathway have failed to identify a role for 5-HT, although 5-HT₃ receptor blockade did depress ascending excitatory reflexes.⁶ In general, sensory neurones synapse with all other functional types of neurones²⁸ whereas interneurones form chains with each other^{29,30} or project to motor neurones. Thus, a divergence in the pathways may occur between the sensory neurone and the first interneurone in the pathway. 5-HT has been shown to be contained in descending interneurones^{31,32} and so it is likely that 5-HT₃ receptor-mediated transmission occurs either between these interneurones, or between interneurones and excitatory motor neurones in the pathway to the circular muscle.

ATP is an inhibitory transmitter to the intestinal smooth muscle where it acts at P2Y receptors.³³ However, in this study, blockade of P2 receptors significantly decreased the contractile response in both muscle layers, indicating a likely role in the excitatory pathway. PPADS can block smooth muscle P2Y receptors, but at low concentrations used in this study (10 μmol L⁻¹) it appears to be selective for neuronal P2X receptors in the ileum.²⁰ It is likely then that P2X receptors, and ATP as a neurotransmitter, are important in at least one class of synapse in the descending excitatory neuronal pathway to the circular and longitudinal muscle layers, possibly before the pathways to the muscle layers diverge. Spencer *et al.*⁹ found that addition of PPADS to the central chamber of a three-chambered organ bath, in which receptor antagonists

would be expected to act predominantly at synapses between interneurons, did not alter the descending contractile response. However, when hexamethonium, which had little effect by itself, was added together with PPADS there was a significant effect of the latter. This suggests that in the descending excitatory pathway, P2 receptors may have a role in transmission between interneurons, which contrasts with the descending inhibitory pathway where they appear to be important for transmission from descending interneurons to inhibitory motor neurons.²⁰ Taken together, these data suggest that in the descending excitatory pathway ATP may act early, before the pathway diverges.

Acetylcholine acting at nicotinic receptors plays a large role in the ascending excitatory pathway^{5,19,34} and in peristalsis²⁴ but has only a minor role in the descending inhibitory pathways.^{5,19,20,34} In the present study, addition of a nicotinic receptor antagonist to the bath had no effect on the CM contraction, but caused an increase in amplitude of the contraction in the LM. A possible explanation for this effect is that blocking nicotinic transmission removes some tonic inhibitory input to the LM. Spencer *et al.*⁹ examined the role of nicotinic receptors in the central chamber of their bath and found that hexamethonium had no effect on the majority of their preparations. Thus, it seems likely that nicotinic receptor-mediated transmission plays little part in the descending pathways supplying the circular muscle. The majority of myenteric neurones are immunoreactive for choline acetyltransferase (ChAT) and are thus likely to be cholinergic.³² This makes the lack of cholinergic transmission in the descending excitatory pathway surprising, but consistent with findings in the descending inhibitory pathway.

Neurotransmission in peristalsis

The descending excitatory reflex has some properties in common with peristalsis (see above). If these two reflexes are related, then reflex propagation in the flat preparation and in the tubular preparation should utilize many of the same neural pathways. It would therefore be expected that addition of either the 5-HT₃ or P2 receptor antagonist would have similar effects in either preparation. This proved not to be the case. P2 receptor blockade failed to affect peristaltic contractions, whereas in the flat preparation it decreased the contraction in both muscle layers by a substantial amount. Blockade of 5-HT₃ receptors had only one statistically significant effect, shortening the propulsive contractions. The threshold for initiation of peristalsis was increased by both PPADS and granisetron,

but in neither case did this reach statistical significance, and the finding that granisetron has little effect on the threshold for peristaltic reflexes is consistent with earlier studies.³⁵ Similarly, blockade of nicotinic receptors profoundly depressed peristaltic reflexes in modified Trendelenburg preparations,²⁴ but had virtually no effect on the descending excitation in an opened preparation. An explanation for these differences may be that the stimuli used in the two preparations are substantially different. In the modified Trendelenburg preparation, the pressure increases are sustained and distributed along the length of the intestine, whereas in the flat preparation used in the current study, the distension was transient and only involved the region immediately around the balloon. Thus, from the results of this study and others,^{9,24} it appears that descending excitation and peristalsis are two different phenomena that are mediated via pharmacologically distinct neural pathways. Where the two intersect may be in the prominent plateau seen during the relaxation phase of the propulsive contractions seen in the modified Trendelenburg study; this was sensitive to granisetron, although not PPADS, and hence may have involved the descending excitatory pathway.

CONCLUSION

In conclusion, the descending excitatory reflex evoked by distension is likely to be composed of neuronal pathways that separate before reaching the motor neurones that supply the different muscle layers. 5-HT is likely to be utilized as a neurotransmitter in the branch of the pathway that supplies motor neurones innervating the circular muscle only, while ATP may be involved in neurotransmission in the pathways to both muscle layers. ACh appears to have little part as a neurotransmitter in either pathway. This pharmacology is in contrast to that of peristalsis and thus the two motor patterns can be distinguished.

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REFERENCES

- 1 Bayliss WM, Starling EH. The movements and innervation of the small intestine. *J Physiol (Lond)* 1899; **24**: 99–143.
- 2 Bayliss WM, Starling EH. The movements and innervation of the small intestine. *J Physiol (Lond)* 1900; **26**: 125–38.

- 3 Bayliss WM, Starling EH. The movements and the innervation of the large intestine. *J Physiol (Lond)* 1900; **26**: 107–18.
- 4 Johnson PJ, Bornstein JC, Burcher E. Roles of neuronal NK1 and NK3 receptors in synaptic transmission during motility reflexes in the guinea-pig ileum. *Br J Pharmacol* 1998; **124**: 1375–84.
- 5 Smith TK, Furness JB. Reflex changes in circular muscle activity elicited by stroking the mucosa: an electrophysiological analysis in the isolated guinea-pig ileum. *J Auton Nerv Syst* 1988; **25**: 205–18.
- 6 Yuan SY, Bornstein JC, Furness JB. Investigation of the role of 5-HT₃ and 5-HT₄ receptors in ascending and descending reflexes to the circular muscle of guinea-pig small intestine. *Br J Pharmacol* 1994; **112**: 1095–100.
- 7 Waterman SA, Tonini M, Costa M. The role of ascending excitatory and descending inhibitory pathways in peristalsis in the isolated guinea-pig small intestine. *J Physiol (Lond)* 1994; **481**: 223–32.
- 8 Spencer N, Walsh M, Smith TK. Does the guinea-pig ileum obey the 'law of the intestine'? *J Physiol (Lond)* 1999; **517**: 889–98.
- 9 Spencer NJ, Walsh M, Smith TK. Purinergic and cholinergic neuro-neuronal transmission underlying reflexes activated by mucosal stimulation in the isolated guinea-pig ileum. *J Physiol (Lond)*, 2000; **522**: 321–31.
- 10 Furness JB, Johnson PJ, Pompolo S, Bornstein JC. Evidence that enteric motility reflexes can be initiated through entirely intrinsic mechanisms in the guinea-pig small intestine. *Neurogastroenterol Motil* 1995; **7**: 89–96.
- 11 Kunze WA, Furness JB, Bertrand PP, Bornstein JC. Intracellular recording from myenteric neurons of the guinea-pig ileum that respond to stretch. *J Physiol (Lond)* 1998; **506**: 827–42.
- 12 Smith TK, Bornstein JC, Furness JB. Convergence of reflex pathways excited by distension and mechanical stimulation of the mucosa onto the same myenteric neurons of the guinea pig small intestine. *J Neurosci* 1992; **12**: 1502–10.
- 13 Bornstein JC, Furness JB, Smith TK, Trussell DC. Synaptic responses evoked by mechanical stimulation of the mucosa in morphologically characterized myenteric neurons of the guinea-pig ileum. *J Neurosci* 1991; **11**: 505–18.
- 14 Bertrand PP, Kunze WA, Bornstein JC, Furness JB, Smith ML. Analysis of the responses of myenteric neurons in the small intestine to chemical stimulation of the mucosa. *Am J Physiol* 1997; **273**: G422–35.
- 15 Brookes SJ, Song ZM, Steele PA, Costa MOL L-1. Identification of motor neurons to the longitudinal muscle of the guinea pig ileum. *Gastroenterology* 1992; **103**: 961–73.
- 16 Brookes SJ, Steele PA, Costa MOL L-1. Identification and immunohistochemistry of cholinergic and non-cholinergic circular muscle motor neurons in the guinea-pig small intestine. *Neuroscience* 1991; **42**: 863–78.
- 17 Costa MOL L-1, Brookes SJ, Steele PA, Gibbins I, Burcher E, Kandiah CJ. Neurochemical classification of myenteric neurons in the guinea-pig ileum. *Neuroscience* 1996; **75**: 949–67.
- 18 Holzer P, Schlueter W, Maggi CA. Ascending enteric reflex contraction. roles of acetylcholine and tachykinins in relation to distension and propagation of excitation. *J Pharmacol Exp Ther* 1993; **264**: 391–6.
- 19 Johnson PJ, Bornstein JC, Yuan SY, Furness JB. Analysis of contributions of acetylcholine and tachykinins to neuro-neuronal transmission in motility reflexes in the guinea-pig ileum. *Br J Pharmacol* 1996; **118**: 973–83.
- 20 Bian X, Bertrand PP, Bornstein JC. Descending inhibitory reflexes involve P2X receptor-mediated transmission from interneurons to motor neurons in guinea-pig ileum. *J Physiol* 2000; **528**: 551–60.
- 21 Galligan JJ, Bertrand PP. ATP mediates fast synaptic potentials in enteric neurons. *J Neurosci* 1994; **14**: 7563–71.
- 22 Johnson PJ, Shum OR, Thornton PD, Bornstein JC. Evidence that inhibitory motor neurons of the guinea-pig small intestine exhibit fast excitatory synaptic potentials mediated via P2X receptors. *Neurosci Lett* 1999; **266**: 169–72.
- 23 Waterman SA, Costa M, Tonini M. Modulation of peristalsis in the guinea-pig isolated small intestine by exogenous and endogenous opioids. *Br J Pharmacol* 1992; **106**: 1004–10.
- 24 Bülbring E, Crema A. Observations concerning the action of 5-hydroxytryptamine on the peristaltic reflex. *Br J Pharmacol* 1958; **13**: 444–57.
- 25 Brookes SJH, Chen BN, Costa M, Humphreys CMS. Initiation of peristalsis by circumferential stretch of flat sheets of guinea-pig ileum. *J Physiol (Lond)* 1999; **516**: 525–38.
- 26 Hennig GW, Costa M, Chen BN, Brookes SJ. Quantitative analysis of peristalsis in the guinea-pig small intestine using spatio-temporal maps. *J Physiol (Lond)* 1999; **517**: 575–90.
- 27 Tonini M, Frigo G, Lecchini S, D'Angelo L, Crema A. Hyoscine-resistant peristalsis in guinea-pig ileum. *Eur J Pharmacol* 1981; **71**: 375–81.
- 28 Pompolo S, Furness JB. Ultrastructure and synaptic relationships of calbindin-reactive, Dogiel type II neurons, in myenteric ganglia of guinea-pig small intestine. *J Neurocytol* 1988; **17**: 771–82.
- 29 Mann PT, Southwell BR, Young HM, Furness JB. Appositions made by axons of descending interneurons in the guinea-pig small intestine, investigated by confocal microscopy. *J Chem Neuroanat* 1997; **12**: 151–64.
- 30 Portbury AL, Pompolo S, Furness JB et al. Cholinergic, somatostatin-immunoreactive interneurons in the guinea pig intestine: morphology, ultrastructure, connections and projections. *J Anat* 1995; **187**: 303–21.
- 31 Furness JB, Costa M. Neurons with 5-hydroxytryptamine-like immunoreactivity in the enteric nervous system: their projections in the guinea-pig small intestine. *Neuroscience* 1982; **7**: 341–9.
- 32 Steele PA, Brookes SJ, Costa M. Immunohistochemical identification of cholinergic neurons in the myenteric plexus of guinea-pig small intestine. *Neuroscience* 1991; **45**: 227–39.
- 33 Matsuo K, Katsuragi T, Fujiki S, Sato C, Furukawa T. ATP release and contraction mediated by different P2-receptor subtypes in guinea-pig ileal smooth muscle. *Br J Pharmacol* 1997; **121**: 1744–8.
- 34 Smith TK, Bornstein JC, Furness JB. Distension-evoked ascending and descending reflexes in the circular muscle of guinea-pig ileum: an intracellular study. *J Auton Nerv Syst* 1990; **29**: 203–17.
- 35 Tuladhar BR, Kaiser M, Naylor RJ. Evidence for a 5-HT₃ receptor involvement in the facilitation of peristalsis on mucosal application of 5-HT in the guinea pig isolated ileum. *Br J Pharmacol* 1997; **122**: 1174–8.