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ROLE OF 5-HT_3 RECEPTORS IN PERISTALTIC REFLEX ELICITED BY STROKING THE MUCOSA IN THE CANINE JEJUNUM

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SUMMARY

1. The role played by the 5-HT_3 receptor, a serotonin subtype receptor, in peristaltic reflexes was studied in dogs first given ketamine, then anaesthetized with urethane (1·0 g kg^{-1}, I.V.) and α-chloralose (100 mg kg^{-1}, I.V.). The jejunal loop was partitioned into two segments with respect to blood supply. Drugs were infused intra-arterially into each segment.

2. Stroking of the mucosa of the aboral and oral segments elicited an ascending contraction and a descending relaxation, respectively.

3. The ascending contraction was concentration-dependently inhibited by treatment of the aboral segment with the 5-HT_3 receptor antagonists ICS 205-930 and ondansetron (1·4 pmol min^{-1} to 14 nmol min^{-1} for both). The maximal inhibition was 49·5 and 69·3%, respectively. The response was not affected by treatment of the oral segment with these drugs. The descending relaxation was inhibited by 51·4 and 60·8%, respectively, by treatment of the oral segment with ICS 205-930 and ondansetron (1·4 nmol min^{-1} for both).

4. The ascending contraction was markedly inhibited by treatment of either segment with hexamethonium (140 nmol min^{-1}). The response was abolished by treating both segments with hexamethonium and by treating the oral segment with atropine (14 nmol min^{-1}).

5. These results suggest firstly that, in the canine jejunum, enteric neurons with 5-HT_3 receptors play a role as sensory neurons or interneurons in the ascending excitatory and the descending inhibitory pathways of the peristaltic reflex elicited by stroking the mucosa, and secondly, that the ascending limb is composed of cholinergic interneurons and motoneurons.

INTRODUCTION

Physiologically, the peristaltic reflex is thought to be induced by chemical substances in the food and digestive secretions, and by mechanical stimulation, such as distension of the gut wall and deformation of the mucosa by the luminal contents. It had been demonstrated that, in the canine small intestine *in vivo*, polarized enteric reflexes that cause a contraction on the oral side of the stimulus and a relaxation on

the aboral side (an ascending contraction and a descending relaxation in the peristaltic reflex) were initiated by stimulation of the mucosa by stroking, or by treatment with acids, bases and spices (Hukuhara, Yamagami & Nakayama, 1958; Ochi, 1959*a*, *b*). It has, more recently, been demonstrated that such polarized reflexes are also initiated in the isolated guinea-pig ileum by mechanical and chemical stimulation of the mucosa (Smith & Furness, 1988; Smith, Bornstein & Furness, 1991; Yuan, Furness, Bornstein & Smith, 1991).

Studies of the enteric reflex in the isolated intestine of the guinea-pig and rat have confirmed that the ascending excitatory pathway is composed of cholinergic interneurons and cholinergic/substance P-containing motoneurons (Grider, 1989; Tonini & Costa, 1990) and that the descending inhibitory pathway is composed of cholinergic interneurons and non-adrenergic inhibitory motoneurons (Grider & Makhlouf, 1986).

Electrophysiological and histochemical studies have confirmed that the interneurons in the descending pathways are cholinergic (Hirst & McKirdy, 1974; Hirst, Holman & McKirdy, 1975; Bornstein, Furness, Smith & Trussell, 1991; Steele, Brookes & Costa, 1991; Brookes, Steele & Costa, 1991) and those in the ascending pathway are also cholinergic in the guinea-pig small intestine (Bornstein *et al.* 1991; Steele *et al.* 1991; Brookes *et al.* 1991). Cholinergic and/or substance P-containing excitatory motoneurons supplying the circular muscle have been identified histochemically (Furness, Lloyd, Sternini & Walsh, 1990; Steele *et al.* 1991; Brookes *et al.* 1991).

It has been shown that serotonin (5-HT) is present in the enteric neurons and enterochromaffin cells of the mucosa (Solica, Capella, Buffa, Usellini, Fiocca & Sessa, 1987; Gershon, Mawe & Branchek, 1989). Studies which suggest that 5-HT acts as a regulator of the peristaltic reflex include those that showed that 5-HT applied to fluid passing through the lumen of the isolated guinea-pig ileum and rabbit jejunum activated the peristaltic reflex (Bülbring & Lin, 1958), and that topical application of 5-HT to the surface of the mucosa initiated an ascending contraction and a descending relaxation in the canine jejunum *in vivo* (Hukuhara, Nakayama & Nanba, 1960).

Previous studies (Neya, Mizutani & Nakayama, 1991; Mizutani, Neya & Nakayama, 1992), which have demonstrated that pharmacological stimulation of 5-HT_3 receptors with 5-HT and 2-methyl-5-HT elicit not only the ascending contraction but also the descending relaxation, suggested that neurons with 5-HT_3 receptors play a role in the ascending and descending pathways as sensory neurons or interneurons. However, the role played by 5-HT_3 receptors in the peristaltic reflex induced by physiological stimulation has not yet been defined.

The aim of the present study was to determine whether 5-HT_3 receptors are involved in the ascending excitatory and the descending inhibitory pathways activated by stroking the mucosa. In addition, the pharmacology of the neuronal connections involved in the ascending contraction elicited by mucosal stimulation were studied.

Some of the results have been presented to the Physiological Society of Japan (Neya, Mizutani, Yamasato & Suga, 1992).

METHODS

Animal preparation

Twenty-nine mongrel dogs of both sexes, weighing 5–13 kg, and in good health, were fasted for 24 h. The animals were anaesthetized with α-chloralose (100 mg kg^{-1}) and urethane (1 g kg^{-1}) administered intravenously after induction with ketamine hydrochloride (10–20 mg kg^{-1}, I.M.). A tracheal cannula was inserted. The femoral artery was cannulated and connected to a pressure transducer (model DX-312, Nihon Koden, Japan) to record systemic blood pressure. The femoral vein was cannulated for systemic administration of lactated Ringer solution (5 ml kg^{-1} h^{-1}), to compensate for loss of body fluid. Vagotomy was carried out by cutting both vagus nerves at the neck. Splanchnicotomy was also undertaken by severing bilaterally and extraperitoneally the major and minor splanchnic nerves and extirpating bilaterally the sympathetic chains from the 13th thoracic to the 4th lumbar ganglia. Denervation of these extrinsic nerves excludes the influence of the extrinsic intestino-intestinal reflex in dogs (Mizutani, Neya & Nakayama, 1990). The end-tidal CO_2 pressure of expired gas was monitored with a capnometer (model 47210A, Hewlett-Packard, USA) and was kept within the range of 33–40 mmHg. The body temperature was maintained at 36–38 °C, using a heating pad placed under the animal and with an infrared lamp. The experiments were carried out in those animals with blood pressures of or above 75 mmHg. The animals were supported by an artificial ventilator (model SN-480-3, Shinano, Japan) via an endotracheal tube with air.

Anaesthesia was maintained by supplemental administration of α-chloralose (100 mg kg^{-1}, I.V.) when the systemic blood pressure and/or end-tidal CO_2 partial pressure of expired gas started to fluctuate. With this schedule, the animals did not respond to noxious stimuli throughout the experiments.

Motility recording

A loop of jejunum 10–12 cm long, with a distribution of blood vessels suitable for the experimental arrangement such as is shown in Fig. 1, was separated from the rest of the jejunum. After the luminal contents of the separated loop were thoroughly washed out with warm physiological saline, a rubber balloon 2·5 cm long was placed at the oral end of the oral segment (Fig. 1), or at the aboral end of the aboral segment. The intraluminal pressure during relaxation of the intestine was set at 5·8 mmHg by adding water to the balloon through a water manometer connected to a pressure transducer (model DX-312, Nihon Koden, Japan). Pressure changes in the balloon due to muscle contractions were recorded with a chart recorder (model Omniace RT2108, Nihondenki San-Ei, Japan).

Mechanical stimulation of the mucosa

Stroking of the mucosal surface was used as a mechanical stimulus of the mucosa to elicit the ascending contraction and the descending relaxation (Hukuhara *et al.* 1958). To stimulate the mucosa, the aboral or oral segment was cut open along its antimesenteric border. The edge of the opened area (3–4 cm long × 2·5–3·0 cm wide) was sewn around a window made in the abdominal wall by cutting out the skin and abdominal muscles. Thus, the mucosal surface of the segment was exposed to view (Fig. 1) and the remaining part was kept in the abdominal cavity. To avoid direct stimulation of the recording segment, the aboral two-thirds of the exposed mucosa was gently stroked by hand with a ball of wet cotton wool (0·5 cm in diameter) for 30 s (Fig. 1). This stimulation was repeated at intervals of more than 20 min.

To determine whether the reflex induced by stroking the mucosa was due purely to the deformation of the mucosa, the circular muscle layer was stroked in the same manner as the mucosa. To do this, the mucosal–submucosal layer of the aboral segment was carefully removed by cutting the fine blood vessels with an electric surgeon's knife (Mera High Power E-11R, Senko Medical Instrument, Japan) to expose the luminal side of the circular muscle layer.

Drug application

The jejunal artery and vein were ligated at the centre of the loop, as shown in Fig. 1. The most orally and aborally situated branches of the jejunal artery in the loop were cannulated with polyethylene tubes (o.d.: 0·8 mm) for drug administration. Drugs were administered intra-

arterially with an infusion pump (model Truth A-II, Nakagawa, Japan) at the rate of 0·14 ml min^{-1}. They reached the mesenteric blood flow within 2 min after the infusion began, due to the dead space of the cannula of 0·21 ml.

The area of distribution of drugs given by intra-arterial infusion was shown by infusion of Methylene Blue (0·1 %) after each experiment. Methylene Blue coloured the segment being infused and invaded the non-infused segment for less than 0·5 cm along the antimesenteric border. In

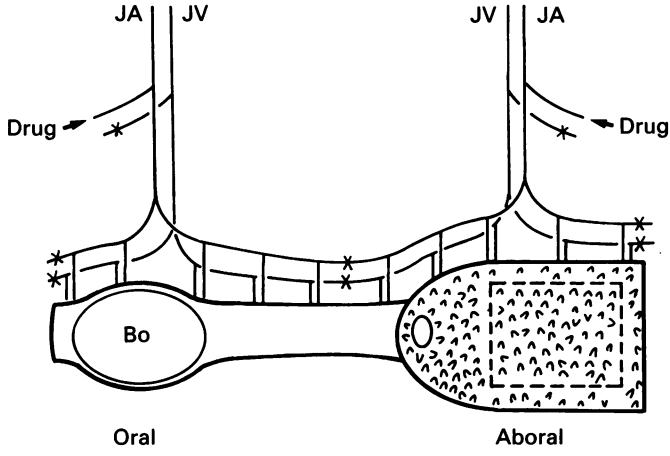

Fig. 1. Arrangement for recording of jejunal motility, mucosal stroking and drug administration. The jejunal loop was neurally decentralized by vagosplanchnicotomy. The oral and aboral segments of the loop were separated with respect to their blood supply by ligation of the jejunal artery (JA) and vein (JV) at the point shown by the crosses. Motility was recorded as pressure change in balloons placed in the oral end (Bo) of the lumen of the loop. The aboral segment was cut along the antimesenteric border and the mucosa was exposed for stimulation by stroking it with a wet cotton ball. The area stroked is shown by the dashed line in the aboral segment. Drugs were administered intra-arterially into either segment from the area shown by arrows. In several experiments, in contrast, the oral segment was used for stroking the mucosa and the anal segment for recording motility.

addition, the dispersion of dye on the mucosal side was seen to cover the same distance as that on the serosal side. The top of the balloon and the oral edge of the stroking area were both at least 1·5 cm from the ligation of the blood vessels. The possibility that drugs administered into the stimulated segment directly affected the motility recording area, and *vice versa*, was therefore excluded.

Drugs were infused at the rate of 0·14 ml min^{-1} for 7 min, before and after infusion of Tyrode's solution at the same rate for 5 min. The mucosa was stimulated 5 min after beginning the infusion of drugs.

Chemicals

Drugs used included atropine sulphate from Merck (Darmstadt, FRG); hexamethonium bromide and nifedipine from Sigma Chemical Co. (St. Louis, MO, USA); tetrodotoxin from Sankyo (Tokyo, Japan); 2-methyl-5-HT and ICS 205-930 (3α-tropanyl)-1H-indole-3-carboxylic acid ester (donated by Sandoz Pharma, Basel, Switzerland); and ondansetron hydrochloride dihydrate (donated by Glaxo Group Research, Greenford). Stock solutions of drugs were prepared in distilled water, with the exception of nifedipine, which was prepared in polyethyleneglycol–ethanol–water (15:15:70, by volume). These solutions were diluted in Tyrode solution just before administration. Intra-arterial administration of Tyrode solution and all solvents produced no significant effect within the used rate and volume.

Data analysis

The contractile response to mucosal stroking was measured as the difference between the maximal pressure developed by the stimulation and the resting pressure level. The relaxation response was also measured as the difference between the minimal pressure of the response and the resting pressure level. The effect of each drug on the responses induced by mucosal stroking was expressed as the percentage of the response level in the presence of the drug to that during the period of control observation. Statistical comparisons were made using Student's standard paired and unpaired t tests. The differences in means were considered significant when $P < 0.05$. All data in the text are expressed as means ± S.E.M., and n represents the number of intestinal loops from which the data were obtained.

RESULTS

Responses to the mucosal stimulation and 5-HT_3 receptor agonist

Stroking the mucosa of the aboral segment elicited a contraction of the oral segment (ascending contraction) and stimulation of the oral segment elicited a relaxation of the aboral segment (descending relaxation) (Fig. 2). 2-Methyl-5-HT (4·4 nmol), a selective 5-HT_3 receptor agonist, injected into the aboral and oral segments elicited the ascending contraction and the descending relaxation, respectively. These responses were reproducible over several hours.

The amplitude of the ascending contraction elicited by the first stimulation of the mucosa, applied at 2 h after the vagosplanchnicotomy, was 8·8 ± 0·6 mmHg (n = 55). The amplitude of the ascending contraction elicited by 2-methyl-5-HT (4·4 nmol) was 9·9 ± 0·8 mmHg (n = 34). When the stimulus was applied to the mucosa of the aboral segment for 30 s at 20 min intervals, ascending contractions with a stable amplitude were reproduced in each loop (Fig. 3*A*), since the fluctuation of the muscle tone in the oral segment was small. Although the amplitude differed between loops, the mean amplitudes in the 2nd to 6th trials were not significantly different from the mean of the 1st trial (Fig. 3*B*).

The descending relaxation in response to stroking of the mucosa was also reproducible. The amplitude of the descending relaxation was significantly smaller (1·8 ± 0·3 mmHg, n = 15) than that of the ascending contraction (Fig. 2), due to the low level of the muscle tone of the aboral segment. The amplitude of each response changed, largely depending on the fluctuation of the muscle tone of the aboral segment. However, a response with stable amplitude was reproducible in the segment whose muscle tone was kept constant.

In the loops in which stroking the mucosa of the aboral segment elicited an ascending contraction in the oral segment (10·2 ± 0·8 mmHg, n = 3), stroking the circular muscle layer of the aboral segment induced no response in the oral segment, even at 120 min after removal of the mucosl–submucosal layer.

Effects of blockade of 5-HT_3 receptors on the ascending contraction

ICS 205-930

Treatment of the aboral segment with ICS 205-930 (1·4 pmol min^{-1} to 14 nmol min^{-1}), an antagonist of 5-HT_3 (Richardson, Engel, Donatsch & Stadler, 1985) and putative 5-HT_4 receptors (Craig & Clarke, 1990; Craig, Eglen, Walsh, Perkins, Whiting & Clarke, 1990), reduced the amplitude of the ascending contraction

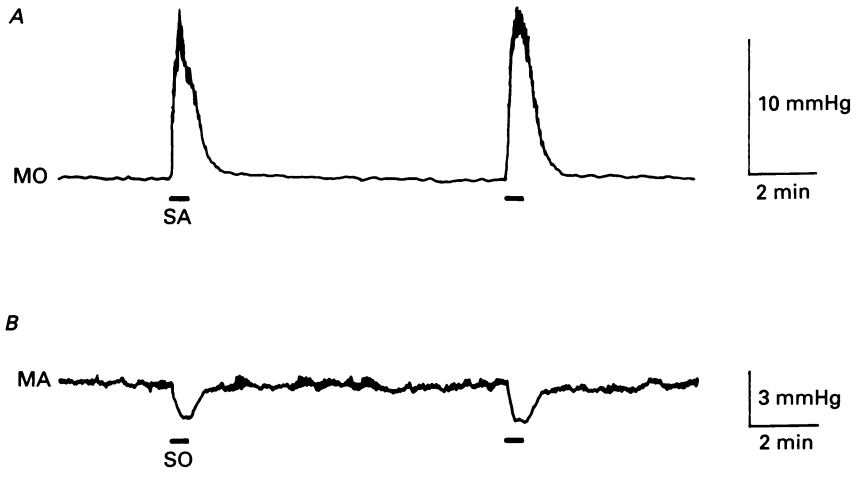

Fig. 2. Peristaltic reflexes elicited by stroking the mucosa. *A*, stroking the mucosa of the aboral segment (SA) produced excitation of the motility of the oral segment (MO), an ascending contraction; *B*, stroking the mucosa of the oral segment (SO) produced an inhibition of motility of the aboral segment (MA), a descending relaxation.

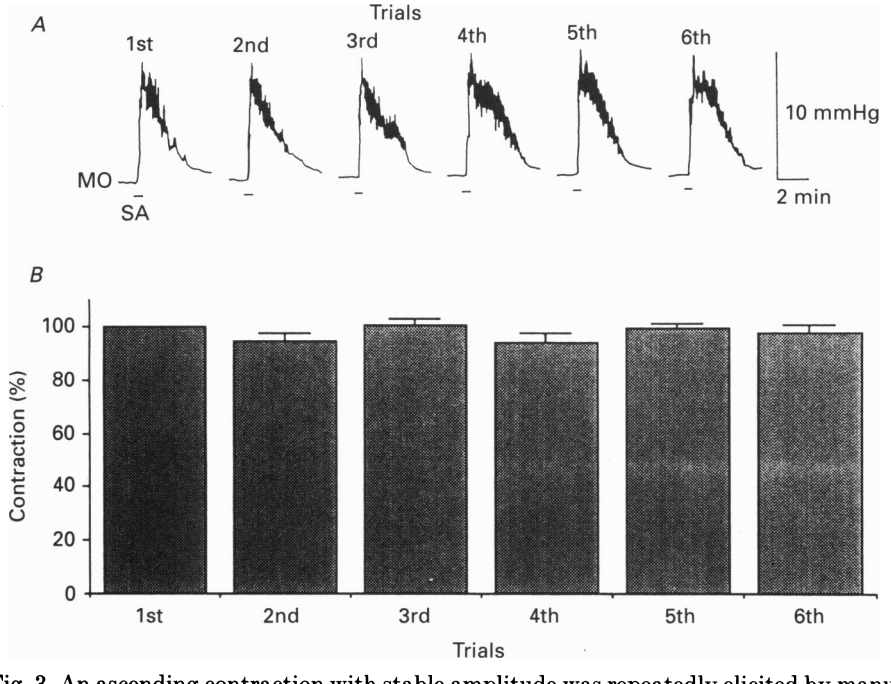

Fig. 3. An ascending contraction with stable amplitude was repeatedly elicited by manual stroking of the mucosa of the aboral segment. Stimuli were applied at intervals of 20 min. *A* shows typical recordings. MO, motility of the oral segment; SA, stroking of the mucosa of the aboral segment. In *B*, the amplitudes of the responses (mean $\pm$ S.E.M., $n = 7$) from the 2nd to 6th trial are expressed as percentages of the response obtained with the first stimulus. Values were calculated from seven complete series of observations.

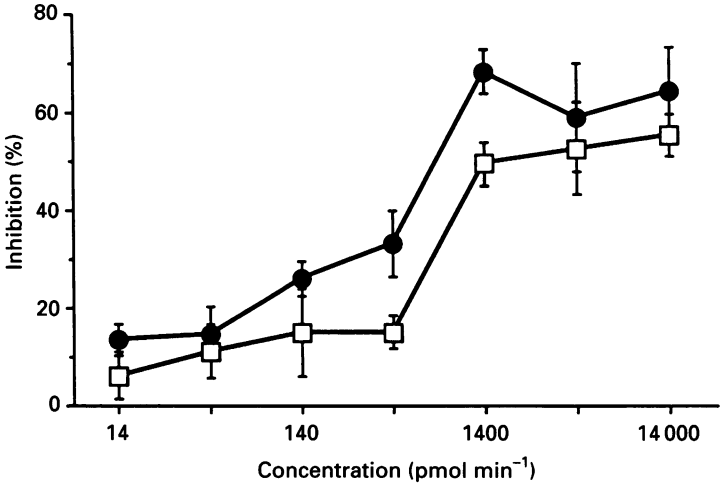

Fig. 4. Concentration-dependent effects of ICS 205-930 (□) and ondansetron (●) infused into the aboral segment against the ascending contraction elicited by stroking the mucosa of the aboral segment. Each value is expressed as the percentage inhibition with these antagonists, and represents mean ± S.E.M., estimated from the data for five loops.

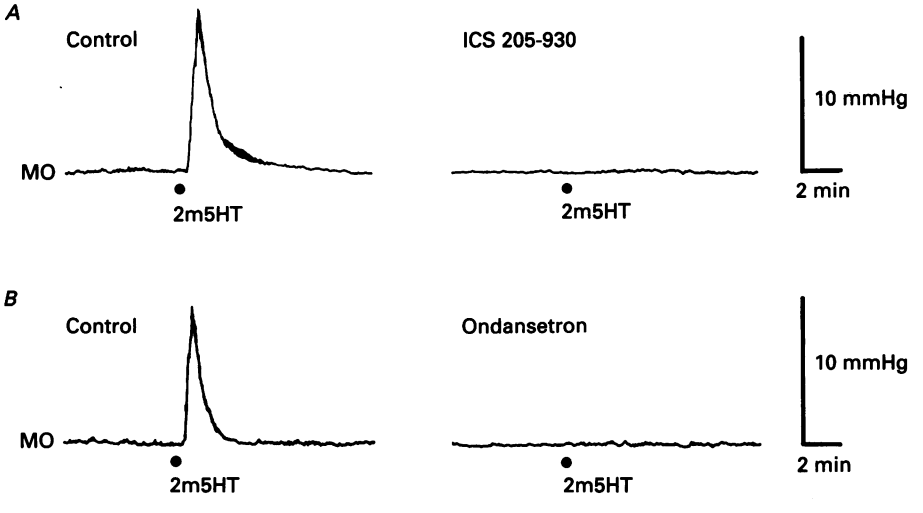

Fig. 5. Effects of ICS 205-930 and ondansetron on the ascending contraction elicited by infusion of 2-methyl-5-HT (2m5HT) into the aboral segment. The responses were abolished by treatment of the aboral segment with ICS 205-930 (1·4 nmol min^{-1}) (*A*) and ondansetron (1·4 nmol min^{-1}) (*B*). MO shows motility of the oral segment.

induced by stroking the mucosa in a concentration-dependent manner (Fig. 4). The inhibitory effect of ICS 205-930 reached its maximum at 1·4 nmol min^{-1} and the amplitude was reduced to 50·5 ± 4·5% ($n = 5$, $P < 0·001$). The half-inhibitory concentration (IC_{50}) of ICS 205-930 was 560 pmol min^{-1}.

ICS 205-930 (1·4 nmol min^{-1}) administered into the oral segment had no inhibitory effect on the ascending contraction elicited by stroking the mucosa (98·0±2·5%, $n = 4$, $P > 0.05$).

Treatment of the aboral segment with ICS 205-930 (1·4 nmol min^{-1}) completely abolished the ascending contraction evoked by 2-methyl-5-HT (4·4 nmol) injected into the aboral segment ($n = 4$) (Fig. 5).

Ondansetron

Treatment of the aboral segment with ondansetron (14 pmol min^{-1} to 1·4 nmol min^{-1}), a selective 5-HT_3 receptor antagonist (Butler, Hill, Ireland, Jordan & Tyers, 1988), also inhibited the ascending contraction elicited by stroking the mucosa in a concentration-dependent manner (Fig. 4). The maximal inhibition was 69·3±4·5% ($n = 5$, $P < 0.001$) at 1·4 nmol min^{-1} ondansetron. The IC_{50} value of ondansetron was 284 pmol min^{-1}.

Ondansetron (1·4 nmol min^{-1}) administered into the oral segment had no effect on the ascending contraction elicited by mucosal stroking (102·0±5·2%, $n = 4$, $P > 0.05$). This concentration of ondansetron completely abolished the ascending contraction evoked by 2-methyl-5-HT (4·4 nmol) in three loops tested (Fig. 5).

Effects of blockade of 5-HT_3 receptors on the descending relaxation

The descending relaxation induced by stroking the mucosa of the oral segment was reduced to 48·6±8·9% ($n = 6$, $P < 0.01$) by treatment of the oral segment with ICS 205-930 (1·4 nmol min^{-1}) (Fig. 6). The response returned to 87·9±4·4% at 40 min after termination of the treatment.

Treatment of the oral segment with ondansetron (1·4 nmol min^{-1}) also reduced the descending relaxation, to 39·2±2·5% ($n = 3$, $P < 0.01$) (Fig. 6). The response returned to 91·6±16·1% at 40 min after termination of the treatment.

Effects of some blockers on the ascending contraction

Tetrodotoxin

Tetrodotoxin (0·43 nmol min^{-1}) administered into the oral and the aboral segments markedly reduced the ascending contraction induced by stroking the mucosa, to 2·2±1·8% ($n = 3$, $P < 0.001$) and 3·9±3·4% ($n = 4$, $P < 0.001$), respectively (Fig. 7).

Nifedipine

The amplitude of the ascending contraction evoked by stroking the mucosa was not reduced significantly (94·2±13·5%, $n = 5$, $P > 0.05$) by nifedipine treatment (14 nmol min^{-1}) of the aboral segment (Fig. 7). In contrast, the ascending contraction was inhibited, to 18·4±3·9% ($n = 3$, $P < 0.001$), when the oral segment was treated with nifedipine (Fig. 7). The contraction was restored to 87·6±13·0% at 40 min after termination of the treatment ($n = 3$, $P > 0.05$).

Hexamethonium

Treatment of the oral segment with hexamethonium (140 nmol min^{-1}) reduced the ascending contraction elicited by mucosal stroking (19·5±6·5%, $n = 4$, $P < 0.01$), and treatment of the aboral segment also reduced the ascending contraction

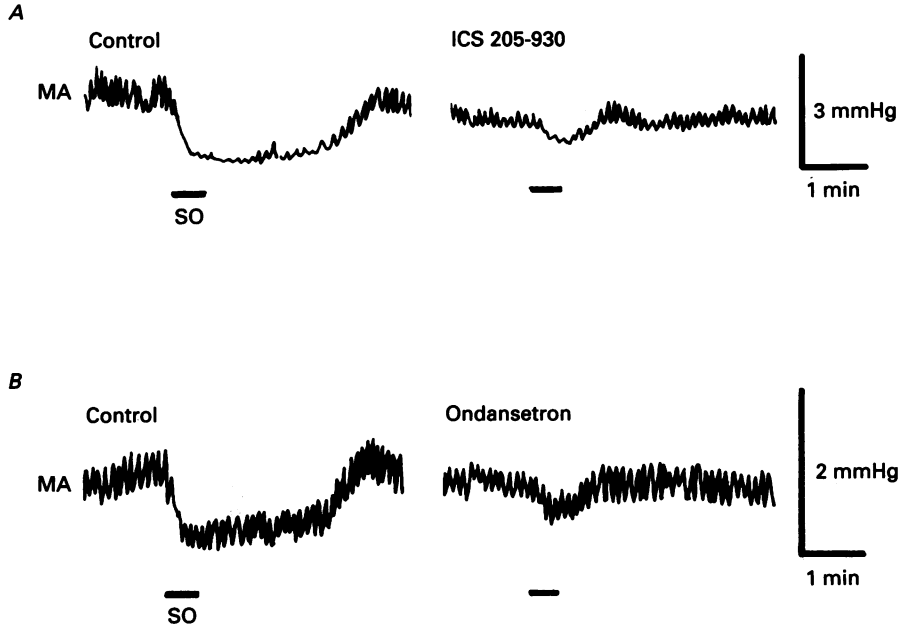

Fig. 6. Effects of ICS 205-930 and ondansetron on descending relaxation. The response elicited in the aboral segment by stroking the mucosa of the oral segment (SO) was markedly reduced by treatment of the stimulated segment with ICS 205-930 (1·4 nmol min^{-1}) (*A*) and ondansetron (1·4 nmol min^{-1}) (*B*). MA shows motility of the aboral segment.

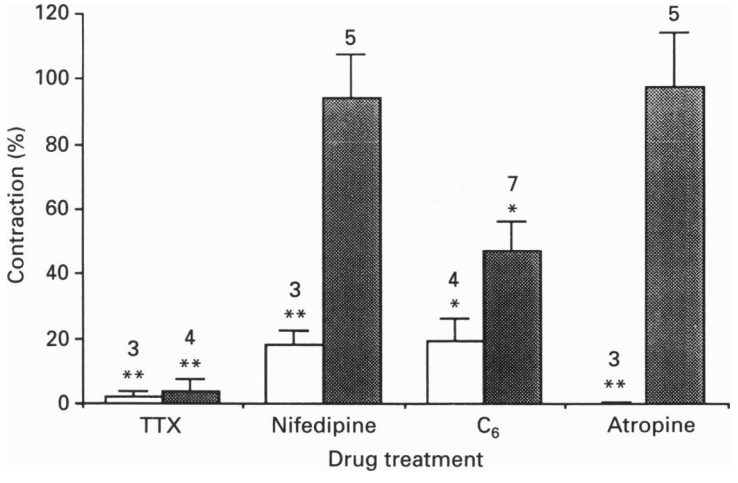

Fig. 7. Effects of tetrodotoxin (TTX, 0·43 nmol min^{-1}), nifedipine (14 nmol min^{-1}), hexamethonium (C_6, 140 nmol min^{-1}) and atropine (14 nmol min^{-1}) on the ascending contraction elicited by stroking the mucosa of the aboral segment. The amplitude of the ascending contraction is expressed as a percentage of the response level obtained before application of drugs. Each value represents mean ± S.E.M. and * and ** indicate $P < 0·01$ and $P < 0·001$, respectively. The number of loops is shown above each column. Oral (□) and aboral (■) indicate treatment of the oral and aboral segments, respectively, with each drug.

(47·2 ± 9·3 %, $n = 7$, $P < 0·01$) (Fig. 7). Treatment of both segments abolished the ascending contraction (4·5 ± 1·8 %, $n = 4$, $P < 0·001$). The response was returned to 70·3 ± 8·4 % ($n = 11$, $P < 0·01$) at 40–60 min after the application of hexamethonium.

Atropine

The ascending contraction elicited by mucosal stroking was abolished by treatment of the oral segment with atropine (14 nmol min^{-1}) (0·2 ± 0·2 %, $n = 3$, $P < 0·001$) (Fig. 7). The response did not return, even at 2 h after administration of atropine. Atropine treatment of the aboral segment caused no inhibition of the ascending contraction (97·7 ± 16·4 %, $n = 5$, $P > 0·05$) (Fig. 7).

DISCUSSION

Selective administration of 5-HT_3 antagonists and several other antagonists through arterial blood flow to the oral and aboral segments of an *in vivo* intestinal loop that had been denervated extrinsically provided advantages in studying the enteric reflex pathway in the dog.

Mucosal stroking is an effective method of stimulation of the enteric reflex in the small intestine both *in vivo* and *in vitro* (Hukuhara *et al.* 1958; Smith & Furness, 1988; Smith *et al.* 1991; Yuan *et al.* 1991). In the present experiments, therefore, we used manual stroking of the mucosa as a stimulus to an ascending contraction and a descending relaxation. The effects of tetrodotoxin and hexamethonium on the ascending contraction confirmed that mucosal stroking activated the enteric neuronal pathways. The finding that no response was elicited by stroking the circular muscle layer of the aboral segment after removal of the mucosal–submucosal layer suggests that a field receptive to the mucosal stimulation was present in the mucosal–submucosal layer.

Our findings that an ascending contraction with a stable amplitude could repeatedly be elicited enabled us to carry out a detailed study of the neuronal mechanism of the ascending contraction evoked by physiological stimulation. By way of contrast, since the loops in which reproducible responses with a stable amplitude in the descending inhibitory pathway occurred were limited in number, determinations of the involvement of 5-HT_3 receptors were limited to the use of a single dose of the antagonists.

Role of 5-HT_3 receptors in the peristaltic reflex

Our previous work (Neya *et al.* 1991; Mizutani *et al.* 1992) indicated that pharmacological stimulation of 5-HT_3 receptors initiated an ascending contraction and a descending relaxation in the canine jejunum. Results in the current study strongly suggest that the 5-HT_3 receptor has a physiological function in the peristaltic reflex in this tissue.

Ondansetron selectively blocks 5-HT_3 receptors (Butler *et al.* 1988), and ICS 205-930 blocks not only 5-HT_3 receptors (Richardson *et al.* 1985) but also putative 5-HT_4 receptors (Craig & Clarke, 1990; Craig *et al.* 1990) in the isolated guinea-pig ileum. Our present findings, that both antagonists inhibited the ascending contraction and the descending relaxation, indicate that mucosal stroking releases endogenous 5-HT

and that 5-HT_3 receptors are involved in both ascending excitatory and descending inhibitory reflexes.

In the guinea-pig ileum, however, it was indicated that 5-HT receptors were not involved in the non-cholinergic excitatory reflex, resistant to hexamethonium and atropine (Tonini, Coccini, Onori, Candura, Rizzi & Manzo, 1992). The non-cholinergic component of the ascending excitatory reflex evoked by inflating an intraluminal balloon was insensitive to different 5-HT antagonists which act at neural 5-HT_1-like, 5-HT_3, 5-HT_4 and putative 5-HT_{1P} receptors. However, it was not the present case, since the ascending reflex in the canine jejunum was blocked completely by treatment of both oral and aboral segments with hexamethonium.

5-HT_3 receptors have been reported to be distributed in the enteric nervous system (Butler *et al.* 1988) and on the enteric neurons (Surprenant & Crist, 1988; Gershon, Wade, Kirchgessner & Tamir, 1990; Frieling, Cooke & Wood, 1991; Wade, Mawe, Branchek & Gershon, 1991) in the guinea-pig intestine. In the present experiments, 5-HT_3 antagonists inhibited the reflex effects when they were used to treat the stroked segment, whereas they had no effect when the motility recording segment was treated. These findings indicate that enteric neurons with 5-HT_3 receptors, located in the aboral and oral segments, respectively, mediate the ascending contraction and the descending relaxation elicited by stroking the mucosa: these findings suggest that these neurons play a role as afferents or interneurons common to the ascending excitatory and the descending inhibitory pathways.

5-HT is contained in the enterochromaffin cells of the mucosal epithelium and myenteric neurons, but none of the submucous neurons are thought to be serotonergic. If the source of endogenous 5-HT released by stroking the mucosa was myenteric neurons, the mucosal stroking should activate only the descending inhibitory pathway since the processes of 5-HT-containing neurons project in the oral-to-anal direction in the myenteric plexus (Furness & Costa, 1982). In the present study, both the ascending and the descending reflexes induced by stroking the mucosa were inhibited by 5-HT_3 receptor antagonists. Therefore, it is unlikely that myenteric serotonergic neurons participate in these reflexes.

If the source of endogenous 5-HT is the enterochromaffin cells, it is probable that sensory neurons in the myenteric or submucous plexus respond to this substance. The secretion of 5-HT from enterochromaffin cells by mechanical stimulation (pressure application) of the mucosa has been demonstrated in guinea-pig ileum (Bülbring & Lin, 1958; Bülbring & Crema, 1959). It was suggested that deformation of the mucosa by puffs of N_2 released 5-HT from the enterochromaffin cells in the guinea-pig ileum (Kirchgessner, Tamir & Gershon, 1992). 5-HT applied to the mucosal surface caused the ascending excitatory and the descending inhibitory reflexes in the canine jejunum (Hukuhara *et al.* 1960). These findings imply that sensory endings of the mucosa for the intrinsic reflexes can be activated by 5-HT released from the enterochromaffin cells. In the guinea-pig ileum, it is thought that, in the circuits mediating the ascending and descending mucosa-to-muscle reflexes, myenteric AH neurons are primary sensory neurons, which project their processes in the mucosa (Bornstein *et al.* 1991). 5-HT_3 receptors are found on AH neurons of the guinea-pig myenteric plexus (Wade *et al.* 1991). In contrast, 5-HT receptors were not identified on submucous AH neurons (Galligan, Surprenant, Tonini & North, 1988),

although 5-HT_3 receptors were identified on submucous S-like neurons (Surprenant & Crist, 1988). Therefore, it seems likely that the neurons with 5-HT_3 receptors (probably myenteric AH neurons) which respond to the mucosal stroking play a role as the sensory neurons rather than the interneurons in our ascending and descending reflex pathways.

In the present experiments, we investigated only the involvement of the 5-HT_3 receptor in the peristaltic reflex elicited by stroking the mucosa. The inability of the 5-HT_3 antagonists to block completely the ascending and descending reflexes suggests that stroking the mucosa can excite these reflex pathways via a mechanism independent of 5-HT_3 receptors. In this respect, the presence has been reported of 5-HT_{1P} receptors on the primary sensory neurons in the submucous plexus which are activated by mucosal deformation with puffs of N_2 in the guinea-pig small intestine (Kirchgessner *et al.* 1992).

Pathways for the ascending contraction

Nifedipine inhibited tonic and phasic contractions of the longitudinal and circular muscles of the guinea-pig ileum by its calcium channel blocking action (Grbovic & Radmanovic, 1987). Our previous study (Mizutani *et al.* 1992) showed that an ascending contraction induced by 5-HT_3 receptor stimulation with 2-methyl-5-HT in the aboral segment was reduced to about 70% when the contraction in the aboral segment was abolished by treatment of this segment with nifedipine. We have proposed a major pathway that is activated by the direct action of 2-methyl-5-HT on the 5-HT_3 receptors on enteric neurons in the aboral segment, and a minor one that is secondarily activated by the contraction of the aboral segment induced by 2-methyl-5-HT. The present finding that nifedipine had no inhibitory effect on the ascending contraction elicited by the mucosal stroking implies that the ascending contraction was not secondarily initiated by the muscle contraction of the aboral segment that might have been induced by the mucosal stimulation.

The interneurons in the ascending excitatory pathway activated by radial stretching in the rat colon and human jejunum (Grider & Makhlouf, 1986; Grider, 1989), and by distension of the wall with a balloon or deforming the mucosa with a brush in the isolated guinea-pig ileum (Tonini & Costa, 1990; Bornstein *et al.* 1991; Smith *et al.* 1991), may be cholinergic. Cholinergic interneurons directed orally have been identified immunohistochemically in the guinea-pig myenteric plexus (Steele *et al.* 1991). In the present experiments, hexamethonium administered into either the oral segment or the aboral segment inhibited the ascending contraction, and administration into both segments abolished the ascending contraction. These findings indicate that the ascending excitatory pathway contains nicotinic synapses in the oral and aboral segments, and thus suggest that interneurons in the ascending pathways are also cholinergic in the canine small intestine.

It has been suggested that the motoneurons of the ascending excitatory pathway activated by mechanical stimulation are cholinergic and substance P-containing in the rat colon (Grider & Makhlouf, 1988), human jejunum (Grider, 1989) and guinea-pig ileum (Tonini & Costa, 1990) since the ascending contraction is reduced by atropine and abolished by tetrodotoxin. Cholinergic and cholinergic/substance P-containing motoneurons have been identified in the guinea-pig small intestine (Steele *et al.* 1991; Brookes *et al.* 1991) and substance P-containing neurons have been

identified in the canine small intestine (Furness *et al.* 1990). In the present results, abolition of the ascending contraction by administration of atropine into the oral segment suggests that, in the canine small intestine, motoneurons for the ascending contraction elicited by stroking the mucosa are cholinergic and that the neuromuscular transmission is mediated by muscarinic receptors. This cholinergic component of the ascending contraction is similar to that activated by the pharmacological stimulation of 5-HT_3 receptors (Mizutani *et al.* 1992).

In conclusion, the results here suggest that peristaltic reflexes elicited by stroking the mucosa are mediated partly by 5-HT_3 receptors on sensory neurons or interneurons that are common to the ascending excitatory and the descending inhibitory pathways, and the results may indicate that the ascending pathway is composed of cholinergic interneurons and motoneurons.

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REFERENCES

Bornstein, J. C., Furness, J. B., Smith, T. K. & Trussell, D. C. (1991). Synaptic responses evoked by mechanical stimulation of the mucosa in morphologically characterized myenteric neurons of the guinea-pig ileum. *Journal of Neuroscience* **11**, 505–518.

Brookes, S. J. H., Steele, P. A. & Costa, M. (1991). Identification and immunohistochemistry of cholinergic and non-cholinergic circular muscle motor neurons in the guinea-pig small intestine. *Neuroscience* **42**, 863–878.

Bülbring, E. & Crema, A. (1959). The release of 5-hydroxytryptamine in relation to pressure exerted on the intestinal mucosa. *Journal of Physiology* **146**, 18–28.

Bülbring, E. & Lin, R. C. Y. (1958). The effect of intraluminal application of 5-hydroxytryptamine and 5-hydroxytryptophan on peristalsis; the local production of 5-HT and its release in relation to intraluminal pressure and propulsive activity. *Journal of Physiology* **140**, 381–407.

Butler, A., Hill, J. M., Ireland, S. J., Jordan, C. C. & Tyers, M. B. (1988). Pharmacological properties of GR 38032F, a novel antagonist at 5-HT_3 receptors. *British Journal of Pharmacology* **94**, 397–412.

Craig, D. A. & Clarke, D. E. (1990). Pharmacological characterization of a neuronal receptor for 5-hydroxytryptamine in guinea pig ileum with properties similar to the 5-hydroxtryptamine$_4$ receptor. *Journal of Pharmacology and Experimental Therapeutics* **252**, 1378–1386.

Craig, D. A., Eglen, R. M., Walsh, L. K. M., Perkins, L. A., Whiting, R. L. & Clarke, D. E. (1990). 5-Methoxytryptamine and 2-methyl-5-hydroxytryptamine-induced desensitization as a discriminative tool for the 5-HT_3 and putative 5-HT_4 receptors in guinea pig ileum. *Naunyn-Schmiedeberg's Archives of Pharmacology* **342**, 9–16.

Frieling, T., Cooke, H. J. & Wood, J. D. (1991). Serotonin receptors on submucous neurons in guinea pig colon. *American Journal of Physiology* **261**, G1017–1023.

Furness, J. B. & Costa, M. (1982). Neurons with 5-hydroxytryptamine-like immunoreactivity in the enteric nervous system: Their projections in the guinea-pig small intestine. *Neuroscience* **7**, 341–349.

Furness, J. B., Lloyd, K. C. K., Sternini, C. & Walsh, J. H. (1990). Projections of substance P, vasoactive intestinal peptide and tyrosine hydroxylase immunoreactive nerve fibres in the canine intestine, with special reference to the innervation of the circular muscle. *Archives of Histology and Cytology* **53**, 129–140.

Galligan, J. J., Surprenant, A., Tonini, M. & North, R. A. (1988). Differential localization of

5-HT_1 receptors on myenteric and submucosal neurons. *American Journal of Physiology* **255**, G603–611.

GERSHON, M. D., MAWE, G. M. & BRANCHEK, T. A. (1989). 5-Hydroxytryptamine and enteric neurons. In *Peripheral Actions of 5-Hydroxytryptamine*, ed. FOZARD, J. R., pp. 247–264. Oxford University Press, Oxford.

GERSHON, M. D., WADE, P. R., KIRCHGESSNER, A. L. & TAMIR, H. (1990). 5-HT receptor subtypes outside the central nervous system. Roles in the physiology of the gut. *Neuropsychopharmacology* **3**, 385–395.

GRBOVIC, L. & RADMANOVIC, B. Z. (1987). The effect of nifedipine on the contractile responses of the longitudinal and circular muscle of the isolated guinea-pig ileum to various agonists. *Archives Internationales de Pharmacodynamie et de Therapie* **289**, 165–176.

GRIDER, J. R. (1989). Tachykinins as transmitters of ascending contractile component of the peristaltic reflex. *American Journal of Physiology* **257**, G709–714.

GRIDER, J. R. & MAKHLOUF, G. M. (1986). Colonic peristaltic reflex: identification of vasoactive intestinal peptide as mediator of descending relaxation. *American Journal of Physiology* **251**, G40–45.

GRIDER, J. R. & MAKHLOUF, G. M. (1988). Regulation of the ascending contraction component of the peristaltic reflex by myenteric tachykinin neurons. *Gastroenterology* **94**, A157.

HIRST, G. D. S., HOLMAN, M. E. & MCKIRDY, H. C. (1975). Two descending nerve pathways activated by distension of guinea-pig small intestine. *Journal of Physiology* **244**, 113–127.

HIRST, G. D. S. & MCKIRDY, H. C. (1974). A nervous mechanism for descending inhibition in guinea-pig small intestine. *Journal of Physiology* **238**, 129–144.

HUKUHARA, T., NAKAYAMA, S. & NANBA, R. (1960). The effects of 5-hydroxytryptamine upon the intestinal motility, especially with respect to the intestinal mucosal intrinsic reflex. *Japanese Journal of Physiology* **10**, 420–426.

HUKUHARA, T., YAMAGAMI, M. & NAKAYAMA, S. (1958). On the intestinal intrinsic reflexes. *Japanese Journal of Physiology* **8**, 9–20.

KIRCHGESSNER, A. L., TAMIR, H. & GERSHON, M. D. (1992). Identification and stimulation by serotonin of intrinsic sensory neurons of the submucosal plexus of the guinea pig gut: Activity-induced expression of fos immunoreactivity. *Journal of Neuroscience* **12**, 235–248.

MIZUTANI, M., NEYA, T. & NAKAYAMA, S. (1990). Capsaicin-sensitive afferents activate a sympathetic intestinointestinal inhibitory reflex in dog. *Journal of Physiology* **425**, 133–144.

MIZUTANI, M., NEYA, T. & NAKAYAMA, S. (1992). Ascending contraction mediated by 5-hydroxytryptamine$_3$ receptors in the canine small intestine. *American Journal of Physiology* **263**, G306–311.

NEYA, T., MIZUTANI, M. & NAKAYAMA, S. (1991). Contractions mediated by enteric 5-hydroxytryptamine (5-HT_3) receptors in the canine small intestine *in vivo*. In *Gastrointestinal Function. Regulation and Disturbances*, vol. 9, ed. KASUYA, Y., TSUCHIYA, M., NAGAO, F. & MATSUO, Y., pp. 61–68. Excerpta Medica, Tokyo.

NEYA, T., MIZUTANI, M., YAMASATO, T. & SUGA, H. (1992). Involvement of 5-HT_3 receptors in the ascending contraction induced by intestinal mucosal stroking. *Journal of the Physiological Society of Japan* **45**, 48 (in Japanese).

OCHI, K. (1959*a*). The substances producing the intrinsic intestinal reflex. I. On the spices. *Journal of the Physiological Society of Japan* **21**, 441–447 (in Japanese).

OCHI, K. (1959*b*). The substances producing the intrinsic intestinal reflex. III. On the acids and bases. *Journal of the Physiological Society of Japan* **21**, 1316–1320 (in Japanese).

RICHARDSON, B. P., ENGEL, G., DONATSCH, P. & STADLER, P. A. (1985). Identification of serotonin M-receptor subtypes and their specific blockade by a new class of drugs. *Nature* **316**, 126–131.

SMITH, T. K., BORNSTEIN, J. C. & FURNESS, J. B. (1991). Interactions between reflexes evoked by distension and mucosal stimulation: electrophysiological studies of guinea-pig ileum. *Journal of the Autonomic Nervous System* **34**, 69–76.

SMITH, T. K. & FURNESS, J. B. (1988). Reflex changes in circular muscle activity elicited by stroking the mucosa: an electrophysiological analysis in the isolated guinea-pig ileum. *Journal of the Autonomic Nervous System* **25**, 205–218.

SOLICA, E., CAPELLA, C., BUFFA, R., USELLINI, L., FIOCCA, R. & SESSA, F. (1987). Endocrine cells of the digestive system. In *Physiology of the Gastrointestinal Tract*, 2nd edn, ed. JOHNSON, L. A., pp. 111–130. Raven Press, New York.

STEELE, P. A., BROOKES, S. J. H. & COSTA, M. (1991). Immunohistochemical identification of cholinergic neurons in the myenteric plexus of guinea-pig small intestine. *Neuroscience* **45**, 227–239.

SURPRENANT, A. & CRIST, J. (1988). Electrophysiological characterization of functionally distinct 5-hydroxytryptamine receptors on guinea-pig submucous plexus. *Neuroscience* **24**, 283–295.

TONINI, M., COCCINI, T., ONORI, L., CANDURA, S. M., RIZZI, C. A. & MANZO, L. (1992). The influence of neuronal 5-hydroxytryptamine receptor antagonists on non-cholinergic ganglionic transmission in the guinea-pig enteric excitatory reflex. *British Journal of Pharmacology* **107**, 5–7.

TONINI, M. & COSTA, M. (1990). A pharmacological analysis of the neuronal circuitry involved in distension-evoked enteric excitatory reflex. *Neuroscience* **38**, 787–795.

WADE, P. R., MAWE, G. M., BRANCHEK, T. A. & GERSHON, M. D. (1991). Use of stereoisomers of zacopride to analyze actions of 5-hydroxytryptamine on enteric neurons. *American Journal of Physiology* **260**, G80–90.

YUAN, S. Y., FURNESS, J. B., BORNSTEIN, J. C. & SMITH, T. K. (1991). Mucosal distortion by compression elicits polarized reflexes and enhances responses of the circular muscle to distension in the small intestine. *Journal of the Autonomic Nervous System* **35**, 219–226.