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5-HT_3 and 5-HT_4 receptor-mediated facilitation of the emptying phase of the peristaltic reflex in the marmoset isolated ileum

[1]B.R. Tuladhar, B. Costall & R.J. Naylor

Postgraduate Studies in Pharmacology, The School of Pharmacy, University of Bradford, Bradford BD7 1DP

1 The patterns of intestinal motility and the effect of an increase in intraluminal pressure were studied *in vitro* on segments obtained from the marmoset small intestine.

2 Segments obtained from the distal half of the marmoset small intestine exhibited segmentation, consisting of narrow annular contractions (lasting for 2–3 s) interposed between the relaxed segments of varying length. The subsequent contractions occurred slightly distal to the previous contraction, with ring-like contractions appearing to move in the aboral direction. Such segmentation was infrequent or absent in the segments obtained from the proximal half of the small intestine. An increase in intraluminal pressure inhibited segmentation and finally produced peristalsis in most of the tissues.

3 The influence of 5-hydroxytryptamine (5-HT) receptor agonists and antagonists on the threshold of the peristaltic reflex was investigated in the segments obtained from the distal half of the intestine after segmentation had subsided. The effect of drug application to the serosal surface was measured as a change in threshold pressure required to trigger the peristaltic reflex. A facilitation was defined in two ways (a) as a reduction in the threshold pressure required to trigger the reflex and (b) in those tissues that failed to respond with peristalsis on raising intraluminal pressure to the maximum attainable (1 kPa), as a reduction in threshold pressure compared to this value.

4 5-HT (7.85±0.19), 5-methoxytryptamine (7.79±0.24), 5-carboxamidotryptamine (6.66±0.13) and 2-methyl-5-HT (6.24±0.16) caused a concentration related facilitation of the peristaltic reflex, the pD_2 values (mean±s.e.mean) being shown in parentheses.

5 The concentration-response curves to both 5-HT and 5-methoxytryptamine were dextrally shifted in a surmountable manner in the presence of GR 113808 (30 nM). pD_2 values for 5-HT and 5-methoxytryptamine were significantly decreased to 6.98±0.24 and 6.83±0.36 respectively in the presence of GR 113808 (30 nM).

6 In the presence of a high concentration of (10 μM) 5-methoxytryptamine the subsequent addition of 2-methyl-5-HT (3–10 μM) but not 5-methoxytryptamine (10 μM) facilitated peristalsis; the effect of 3 μM 2-methyl-5-HT was significantly decreased by 2 μM ondansetron.

7 It is concluded that the facilitation of the peristaltic reflex in the marmoset intestine induced by 5-HT at submicromolar concentrations involves a 5-HT_4 receptor stimulation with an additional 5-HT_3 receptor activation at higher concentrations.

Keywords: 5-Hydroxytryptamine; 5-HT_3/5-HT_4 receptors; marmoset ileum; segmentation; peristaltic reflex; GR 113808

Introduction

Segmentation, peristalsis and pendular movements constitute the essential elements from which complex patterns of motility are constructed in the small intestine (Wingate, 1983). Segmentation and peristalsis are mainly the result of circular muscle activity whereas pendular movement results from the periodic contraction and relaxation of the longitudinal muscle.

Studies on the mechanisms mediating intestinal motility patterns have focussed on peristalsis. An *in vitro* study of the peristaltic reflex was first carried out by Trendelenburg (1917) using the guinea-pig isolated ileum. The peristaltic reflex in this species was shown to consist of regular and coordinated contractions of both longitudinal and circular muscles. With the distension of the ileum caused by an increase of intraluminal pressure, the preparation responds with an increasing longitudinal muscle contraction which is followed, as the pressure reaches a threshold point, by an aborally directed circular muscle contraction which propels the contents in the anal direction. The phase of longitudinal muscle contraction has been termed a preparatory phase and the circular muscle contraction, an emptying phase (Trendelenburg, 1917).

5-Hydroxytryptamine is found in the gastrointestinal mucosa (Erspamer & Asero, 1952) and in the enteric neurones (Gershon *et al.*, 1965) with a potential to modify gastrointestinal motility, the response to 5-HT varying between species (Costall & Naylor, 1990). Using the isolated guinea-pig ileum, 5-HT applied to the mucosal or serosal surface causes respectively either a facilitation or an inhibition of the peristaltic reflex (Bülbring & Crema, 1958). In *in vivo* experiments, a stimulant action of exogenous 5-HT on intestinal motility has been documented in the dog (Ormsbee *et al.*, 1981) and in the guinea-pig (Bülbring & Crema, 1959), whereas human studies have shown both a stimulant and an inhibitory effect on motility (Hendrix *et al.*, 1957; Schmid & Kinzlmeier, 1959).

Four major subtypes of 5-HT receptors exist within the gastrointestinal tract (5-HT_1, 5-HT_2, 5-HT_3 and 5-HT_4) and have been shown to mediate contraction or relaxation responses in various isolated tissue preparations obtained from the guinea-pig and rat (Bradley *et al.*, 1986; Costall & Naylor, 1990; Dhasmana *et al.*, 1993). However, it is difficult to correlate contractions or relaxations of individual muscle groups with motility patterns, which is a complex phenomenon involving integrated responses to both contractions and relaxations of different smooth muscle groups over time. In this respect, it has been shown that stimulation of the 5-HT_4 receptor increases longitudinal muscle contraction (Buchheit & Buhl, 1991) and decreases the threshold for the initiation of the

[1] Author for correspondence.

circular muscle contraction during peristalsis in the guinea-pig isolated ileum (Craig & Clarke, 1991; Costall *et al.*, 1993). The 5-HT_3 receptor has also been shown to increase the ascending excitatory effect but not the descending inhibitory effect in the guinea-pig isolated ileum (Yuan *et al.*, 1994), whereas Neya *et al.* (1993) report that 5-HT_3 receptor stimulation enhances both the ascending excitatory and descending inhibitory effects in the infused canine jejunum. Whilst the presence of the 5-HT_3 receptor antagonist, tropisetron or ondansetron does not affect the threshold for peristalsis in the guinea-pig isolated ileum (Craig & Clarke, 1991; Costall *et al.*, 1993), this does not exclude a 5-HT_3 receptor involvement in the peristaltic reflex since a higher concentration of 5-HT has been shown to reduce the threshold for peristalsis by stimulation of a 5-HT_3 receptor (Tuladhar *et al.*, 1995).

The guinea-pig has been the species of choice to investigate the peristaltic reflex and the involvement of 5-HT_3 and 5-HT_4 receptors. To the authors' knowledge no study has investigated the peristaltic reflex in the primate. Therefore, the present study was designed to investigate the *in vitro* motility patterns of the marmoset isolated ileum and assess the effects of 5-HT_3 and 5-HT_4 receptor agonists and antagonists on the emptying phase of the peristaltic reflex. Some of these results were presented at the Spring Meeting of the British Pharmacological Society, Manchester, 13–15 April 1994.

Methods

Animals and housing conditions

Common marmosets (*Callithrix jacchus*, University of Bradford bred) of either sex weighing 300–400 g and 18–36 months of age were used. The animals were maintained in a constant environment of 25±1°C with 55±5% humidity. The light cycle consisted of white illumination (fluorescent strip lights), between 07 h 00 min and 19 h 00 min, and red illumination (60 W red light) between 19 h 00 min to 07 h 00 min. A simulated 'dawn' and 'dusk' period was provided by illuminating a single white light bulb (60 W) for 30 min prior to lights on and 30 min following lights out.

The animals were fed twice daily on all days except Tuesdays and Fridays. The morning feed on most days consisted of Mazuri primate diet (SDS Ltd., Essex, England) mixed with water to form a 'mash'. A small amount of condensed milk (Nestlé, Vevey, Switzerland) was added to the mash to increase palatability. The animals were able to feed from this *ad libitum* throughout the day. The second feed, which consisted of a selection of fresh fruit (apples, oranges and bananas), dried fruit (currents and raisins), nut, wholemeal bread or malt loaf, was given at approximately 16 h 00 min.

On Tuesdays and Fridays animals were given a single feed made up of a selection of fruit, bread, malt loaf and scrambled egg and a mash consisting of: 375 g Casilan (milk protein) (Crookes Health Care, Nottingham, England), 340 g Farex (Farley Health Products, Nottingham, England), 114 g Build-up (dietary supplement) (Societe des Produits Nestlé S.A., Vevey, Switzerland), 58 ml Abidec (multi vitamin syrup) (Warner Lambert Health Care, Hampshire, England), 175 ml Cytacon B_{12} (Ducan Flockhart and Co. Ltd., Greenford, England), 300 mg Rovimix (vitamin D_3) (Roche Products, Welwyn Garden City, England), 150 ml natural yogurt and water to mix. Fresh drinking water was available from drinking bottles *ad libitum* throughout the day on all days.

Preparation of the isolated tissues

Twenty-two marmosets were used for the studies. Anaesthetized (1.8 ml kg^{-1} intramuscular Saffan Glaxovet, Uxbridge, UK) animals were killed by decapitation and the small intestine removed and kept in Krebs-Henseleit solution oxygenated with 95% O_2 and 5% CO_2 kept at room temperature. The mesentery was removed and the lumen flushed of its contents, taking care to reduce distension of the ileum to the minimum before mounting the tissue in the bath. The tissues were mounted in the baths within 60 min of decapitation.

Segmentation and peristalsis

The organ bath arrangement used was a modification of the equipment previously described for the guinea-pig ileum (Costall *et al.*, 1993) and a schematic diagram of the apparatus is shown in Figure 1. Briefly, the first outlet (A) connected to the aboral tube had the opening at the level of the fluid in the bath; another outlet (B) consisted of a bent narrow tube of 3 mm internal diameter with an opening at 10 cm above the level of the fluid in the bath. Any volume expelled from this outlet was collected in a 5 ml syringe connected to a pressure transducer (Type 4-422-0001, Bell & Howell Ltd., England) so that the volume expelled could also be recorded. The intraluminal pressure was measured from the aboral side by a second pressure transducer and recorded using a Grass polygraph. The changes in motility patterns were also recorded on video tape.

The Krebs-Henseleit solution (composition mM: NaCl 118, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25 and glucose 10) oxygenated with 95% O_2 and 5% CO_2 was introduced into the lumen of the ileum by use of a Watson-Marlow peristaltic pump at a constant flow rate of 0.5 ml min^{-1}. During the resting or equilibration period the outlet (A) at the level of the fluid in the bath was left open. To examine the effect of drugs on peristalsis, this outlet was closed which resulted in a slow filling of the lumen with distension and a corresponding rise in the fluid level in the second outlet tube. Agonists were added either 1–3 min after the fluid started overflowing from the top outlet or at the end of the emptying phase in tissues undergoing continuous peristalsis. In a few initial experiments designed to study the effect of an increased intraluminal pressure on the segmentation and induction of peristalsis, Krebs-Henseleit solution was introduced by gravity feed from the oral side by raising the reservoir kept on an adjustable stand. The height of outlet (B) was also altered as required in these experiments.

Experimental design for studying the effects of 5-HT receptor agonists and antagonists on peristalsis

The tissues were allowed to equilibrate for at least 4 h with washout every 30 min before priming the tissues with 0.1 μM 5-HT; 15 min after the washout of the priming concentration of 5-HT, concentration-response curves to 5-HT or other agonist were constructed in a noncumulative manner, with at least a 15 min resting period between the doses. The outlet A was left open during the resting period with a continuous flow of

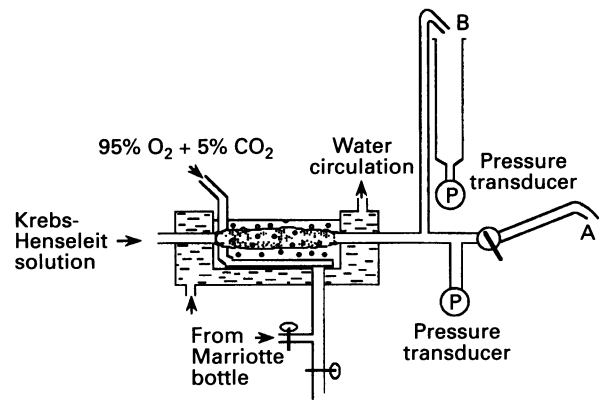

Figure 1 Diagram of the apparatus used to study the emptying phase of the peristaltic reflex in the marmoset isolated ileum.

Krebs-Henseleit solution at a rate of 0.5 ml min^{-1}. Only two terminal tissues (5–6 cm long taken 1–15 cm from the ileo-caecal junction) were removed from each animal and each tissue was used to construct only one concentration-response curve. The Krebs-Henseleit solution passing through the lumen did not contain any drug. GR 113808 was added to the reservoir suppling the bath (serosal side) and equilibrated for at least 1 h before construction of the concentration-response curves.

The peristaltic threshold is defined as the intraluminal pressure required to initiate the peristaltic reflex. In some tissues a control threshold for peristalsis was determined by increasing the intraluminal pressure and noting the threshold for peristalsis in the peristaltic stroke just before the addition of drug. A facilitation of the peristaltic response was defined as any decrease in the threshold for peristalsis in the peristaltic stroke just before the addition of drug and expressed as a percentage of the latter value. The addition of drug immediately after a peristaltic stroke and during a period of regular peristalsis ensured that the immediate or delayed effects of any one concentration of drug could be ascertained on the subsequent strokes, with respect to mono- or biphasic changes in the peristaltic threshold.

In some tissues an increase in intraluminal pressure to the maximum attainable, i.e. 1 kPa, the height of buffer in the top outlet before overflow, failed to induce peristalsis. In such experiments a facilitatory response was defined as a decrease in the threshold for peristalsis expressed as a percentage of 1 kPa. Whilst such measurements do not reflect the absolute reduction in peristaltic threshold, since peristalsis was not actually induced in the absence of drug treatment, the percentage change reflects the minimum change that could have occurred.

The effect of a single higher concentration of 2-methyl-5-HT in the presence and absence of ondansetron was tested on some tissues previously treated with 10 μM 5-methoxytryptamine. 5-Methoxytryptamine was retained in the organ bath in these experiments.

Analysis of results

The ability of the agonists to facilitate peristalsis was expressed as pD_2 values (relative to individual maxima) except when a single concentration of an agonist was tested. pD_2 values were determined graphically from individual concentration-response curves. All results are expressed as arithmetic mean ± s.e.mean. The significance of differences between the values was determined at $P<0.05$ by Student's t test (unpaired one tail) or ANOVA followed by Dunnett's t test.

Drugs

5-Hydroxytryptamine maleate, 5-methoxytryptamine hydrochloride, acetylcholine chloride (Sigma), 2-methyl-5-hydroxytryptamine hydrochloride (Cookson Chemicals Ltd.), ondansetron hydrochloride dihydrate, GR 113808 ([1-[2-methylsulphonyl) amino] ethyl] - 4 - piperidinyl] methyl-1-methyl-1H - indole - 3 - carboxylate) (Glaxo) and 5 - carboxamidotryptamine maleate (RBI) were dissolved in distilled water and diluted in Krebs-Henseleit solution. All drugs were applied to the serosal side and agonists were added in a volume of 250 μl to a bath volume of 25 ml.

Results

General observations

The small intestine of the common marmoset measured 30–40 cm ($n=22$) from the pyloric sphincter to the ileo-caecal junction. The use of fresh segments of intestine from the distal half exhibited segmentation which were infrequent or absent in the proximal tissues ($n=11$). Analysis of video recordings indicated that segmentations consisted of narrow annular contractions interposed between the relaxed segments of varying length (1–4 cm) with minimum length of about 0.5 cm. Each contraction lasted for about 2–3 s. The subsequent contractions occurred slightly more distal to the previous contraction, so that ring-like contractions appeared to move slowly in the aboral direction. The segmentation in any one tissue lasted for 2–4 h. The segmentation response and associated contractions caused highly characteristic changes in intraluminal pressure tracings and was abolished by increasing the intraluminal pressure to 0.1–0.3 kPa ($n=7$) (Figure 2a).

In other experiments, where the threshold for peristalsis was first examined by raising the intraluminal pressure at a rate of 50 Pa min^{-1}, out of seven tissues obtained from the two most distal sections, 6 out of 7 tissues exhibited peristalsis with an increase of the intraluminal pressure with a mean threshold of 884 ± 107 and 576 ± 83 Pa respectively. In the one distal tissue in which an increase of intraluminal pressure (1.2 kPa) failed to induce peristalsis, 0.1 μM 5-HT was able to produce peristalsis. A typical tracing of the induction of the peristaltic reflex with the increase in intraluminal pressure is shown in Figure 2b.

A study of the peristaltic reflex was made over 4 h, during which time segmentation had subsided in all tissues and in most of the tissues and for most of the time no peristalsis occurred in response to the slow increase in intraluminal pressure to a maximum of 1 kPa. Only rarely did tissues exhibit continuous peristalsis with a stable threshold below 1 kPa. The priming concentration of 5-HT (0.1 μM) facilitated peristalsis (in tissues not undergoing peristalsis) or decreased the threshold for peristalsis in all tissues obtained from the distal half of the marmoset small intestine. Therefore, the effect of 5-HT receptor agonists alone or in the presence of antagonists were studied in the tissues obtained from the distal half (1–15 cm from the ileo-caecal junction) of the marmoset small intestine.

As the marmosets received a different diet on Tuesdays and Fridays, we examined whether there was any difference in the

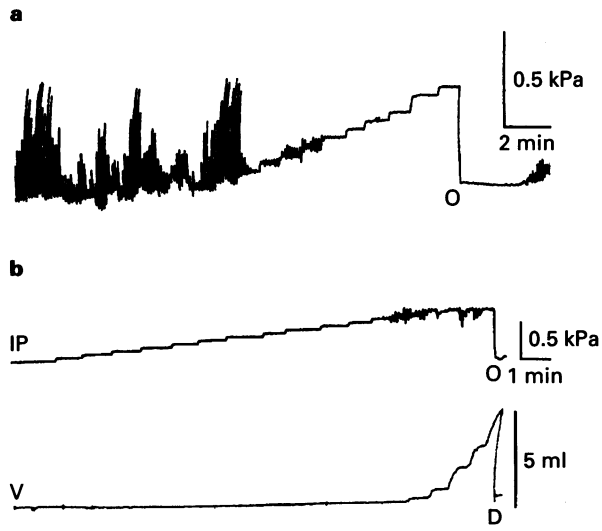

Figure 2 (a) Intraluminal pressure tracing showing an inhibition of the segmentation activity by the increase of the intraluminal pressure in a step wise manner at a rate of 50 Pa min^{-1}. The fall in pressure at the point indicated by O on the intraluminal pressure tracings represents the point where the lower outlet was opened. (b) An example of a tracing showing induction of the peristaltic reflex in the marmoset ileum induced by an increase of the intraluminal pressure at a rate of 50 Pa min^{-1}. The outlet in this experiment was at 7 cm above the fluid in the bath (see Figure 1). IP and V represent respectively the intraluminal pressure tracing and volume expelled from the top outlet tube. O on the intraluminal pressure tracing represents the point where the lower outlet was opened and D on the volume tracing represents the point where the liquid in the syringe was emptied manually.

response (to 0.1 μM 5-HT used as the priming concentration) in the tissues obtained from animals obtained on different days. The results indicated that there was no discernible variation in the response on different days of testing.

The effect of 5-HT and other agonists

The addition of 5-HT (0.01 – 10 μM), 5-methoxytryptamine (0.01 – 10 μM), 5-carboxamidotryptamine (0.1 – 10 μM) and 2-methyl-5-HT (1 – 10 μM) to the tissues caused a concentration-related facilitation of peristalsis. In most of the tissues this effect was characterized by the facilitation of peristalsis in those tissues failing to undergo peristalsis when challenged with the maximum increase in intraluminal pressure. The subsequent peristalsis occurred at a lowered threshold. A typical response to 5-HT is presented in Figure 3. In the tissues undergoing peristalsis the threshold for peristalsis after addition of the 5-HT agonists was again decreased. The maximum responses to the four indole 5-HT receptor agonists were not significantly different.

The potencies of the 5-HT receptor agonists in facilitating the peristaltic reflex in the marmoset isolated ileum are presented in Table 1 and concentration-response curves are presented in Figure 4.

The reduction in the threshold lasted for the whole of the observation time of 5 min at 0.1 and 1 μM of 5-HT ($n=6$). However, at 10 μM 5-HT the facilitatory effect had a shorter duration of 3.5 ± 0.5 min ($n=6$). An inhibitory effect became prominent at 30 μM with only one or two strokes of peristalsis occurring after the addition of 5-HT ($n=6$). However, if the intraluminal pressure was left raised without washing of the tissues, then nearly regular peristalsis usually occurred after a further 20 – 30 min period. The inhibitory effect was clearly evident when 30 μM of 5-HT was added to the tissues undergoing regular peristalsis after the addition of 10, 30 or 100 μM of 5-methoxytryptamine and/or 2-methyl-5-HT ($n=5$, data not shown).

The effect of GR 113808 on concentration-response curves to 5-HT and 5-methoxytryptamine

Both the concentration-response curves to 5-HT and 5-methoxytryptamine to facilitate the peristaltic reflex were shifted dextrally in the presence of GR 113808 (30 nM) with no reduction of the maximum responses. The $pD_2 \pm$ s.e.mean values were for 5-HT without (7.85 ± 0.19, $n=6$) and with GR 113808 (6.98 ± 0.24, $n=3$) (EC_{50} ratio 7.4) and 5-methoxytryptamine without (7.79 ± 0.24, $n=4$) and with GR 113808 (6.83 ± 0.36, $n=4$) (EC_{50} ratio 9.1), showing for both treatments a significant decrease from the control values ($P<0.05$, Student's t test, unpaired one tail). Taking the EC_{50} ratio as the concentration-ratio, apparent pK_B values of 8.32 and 8.43 were calculated for GR 113808 using 5-HT and 5-methoxytryptamine respectively.

Effect of 2-methyl-5-HT in the tissues previously treated with 10 μM 5-methoxytryptamine

2-Methyl-5-HT (3 – 10 μM, $n=4$) but not 5-methoxytryptamine (10 μM, $n=4$) was able to facilitate peristalsis in tissues previously exposed to 10 – 100 μM 5-methoxytryptamine. The facilitatory effect of 3 μM 2-methyl-5-HT in the 5-methoxytryptamine-treated tissues (10 μM for at least 45 min) was significantly reduced by ondansetron (2 μM) ($n=4$) (Figure 5). The antagonistic effect of ondansetron was removed by washout when tested 45 min later.

The effect of acetylcholine

Acetylcholine (ACh) did not stimulate peristalsis at any concentration tested (0.1 μM – 3 mM) ($n=6$, data not shown). ACh actually inhibited peristalsis from 0.1 to 10 μM as was evident when ACh was added to the tissues undergoing peristalsis on its own or after stimulation with 5-HT ($n=3$). At a high concentration (0.3 – 3 mM), ACh caused generalized spasm of whole circular muscle.

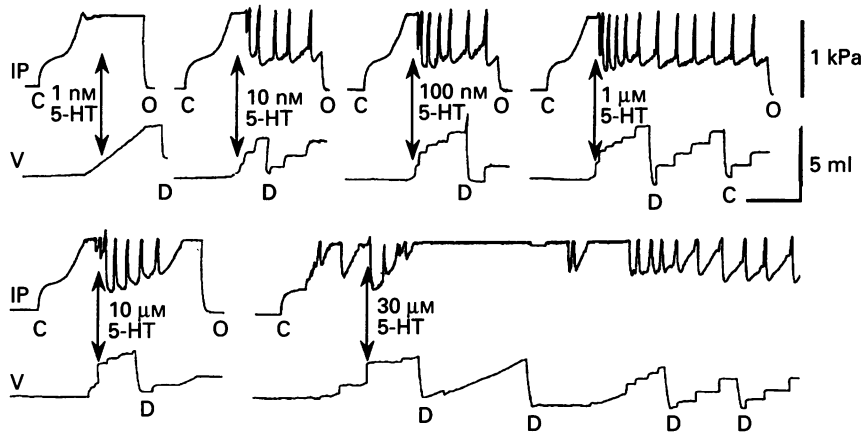

Figure 3 A representative tracing of the effect of 5-HT to induce (10 nM – 30 μM) or induce then block (10 and 30 μM) peristalsis in the marmoset isolated ileum. IP and V represent respectively the intraluminal pressure tracing and volume expelled from the top outlet tube (see Figure 1). C and O on the intraluminal pressure tracings represent the points where the lower outlet was closed and opened respectively. D on the volume tracing represents the point where the liquid in the syringe was emptied manually. Arrows indicate the point of addition of 5-HT. N.B. There was a 15 min delay between opening of the lower outlet (indicated by O) and closing of the lower outlet (indicated by C) for the next cycle.

Table 1 Potency of the 5-HT receptor agonists in facilitating the peristaltic reflex in the marmoset isolated ileum

Agonists	$pD_2 \pm$ *s.e.mean*	n	E_{max}	EC_{50} *ratio*
5-Hydroxytryptamine	7.85 ± 0.19	6	100	1
5-Methoxytryptamine	7.79 ± 0.24	4	112	1.1
5-Carboxamidotryptamine	6.66 ± 0.13	3	78	15.5
2-Methyl-5-HT	6.24 ± 0.16	2	116	40.7

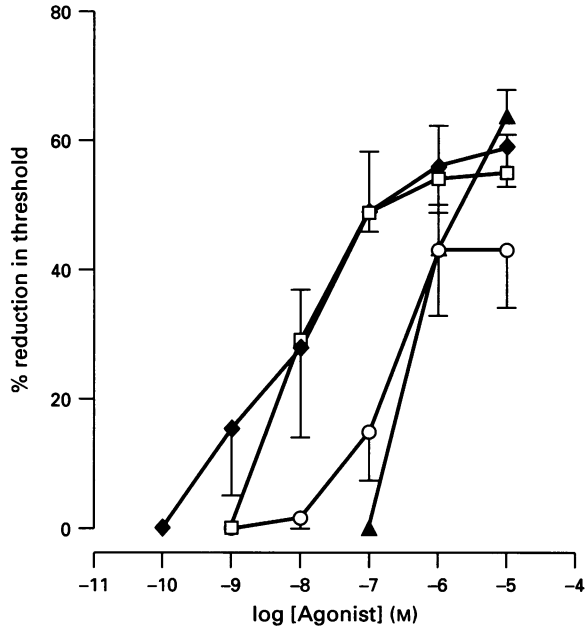

Figure 4 Concentration-response curves to 5-HT (□), 5-methoxytryptamine (◆), 5-carboxamidotryptamine (○) and 2-methyl-5-hydroxytryptamine (▲) for facilitation of the peristaltic reflex in the marmoset isolated ileum. The reduction in threshold is expressed as the percentage reduction from the threshold for peristalsis in the peristaltic stroke just before the addition of drug (in tissues undergoing peristalsis) or below the pressure attained when the fluid was overflowing from the top outlet (1 kPa). The values shown are the mean with s.e.mean ($n=6$, 4, 3 and 2 respectively).

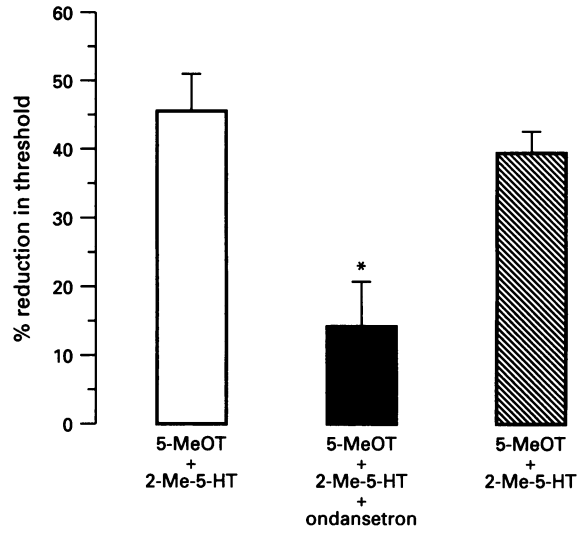

Figure 5 Antagonism by ondansetron of the facilitatory effect of 2-methyl-5-HT (2-Me-5-HT) on peristalsis in the marmoset isolated ileum achieved in the presence of 5-methoxytryptamine (5-MeOT, 10 μM). The facilitation of the peristaltic reflex by 2-methyl-5-HT (3 μM) in the control tissue (open column), in the presence of ondansetron (2 μM) (solid column) and after washout of ondansetron in the latter tissue (hatched column). The reduction in threshold is expressed as the percentage reduction from the threshold for peristalsis in the peristaltic stroke just before the addition of drug (in tissues undergoing peristalsis) or below the pressure attained when the fluid was overflowing from the top outlet (1 kPa). The values shown are the mean with s.e.mean ($n=4$). *Significant difference from the control, $P<0.01$; ANOVA followed by Dunnett's t test.

Discussion

In this study it has been shown that the marmoset ileum exhibits both peristalsis and segmentation patterns of motility. The pattern of motility during segmentation was characterized by annular contractions at a variable distance in the ileum with video recordings clearly demonstrating that subsequent annular contractions travelled in the aboral direction. This is the major difference we have noted from the description of segmentation presented in the pioneering work of Cannon (1902) and may relate to a species difference; Cannon used the cat. The segmentation occurred at low intraluminal pressures and was inhibited by an increase in the intraluminal pressure.

The present study has also demonstrated in the marmoset ileum that exogenous 5-HT at a low concentration can reduce the threshold for peristalsis irrespective of whether the tissue has an on-going peristalsis or not. Importantly, a higher concentration of 5-HT also inhibited peristalsis. In this context the effect of 5-HT in facilitating or inhibiting peristalsis was similar to that seen in the guinea-pig ileum (Kosterlitz & Robinson, 1957; Bülbring & Crema, 1958; Costall *et al.*, 1993; Tuladhar *et al.*, 1995).

In an attempt to characterize the 5-HT receptor mediating the facilitatory effect, the rank order of potency of four indole agonists tested, 5-HT ≥ 5-methoxytrptamine > 5-carboxamidotryptamine > 2-methyl-5-HT is in close agreement with their rank order of potency for the 5-HT_4 receptors in the myenteric plexus of the guinea-pig ileum, the rat tunica muscularis mucosa and the mouse colliculi neurones (Dumuis *et al.*, 1988; Craig *et al.*, 1990; Baxter *et al.*, 1991). This indicates that the facilitatory effect of 5-HT, at least at a concentration below 1 μM, is probably mediated by a 5-HT_4 receptor (Ford & Clarke, 1993; Hoyer *et al.*, 1994). The rank order of potency also correlates with their potency for the 5-HT_4 receptor enhancing the peristaltic reflex in the guinea-pig isolated ileum (Costall *et al.*, 1993). In the latter study the most direct evidence for a 5-HT_4 receptor involvement in peristalsis came from the antagonism of the effect of 5-HT by the 5-HT_3/5-HT_4 receptor antagonist, SDZ 205 557.

In the present study, the ability of GR 113808 at a concentration of 30 nM to shift the concentration-response curve to both 5-HT and 5-methoxytryptamine in a surmountable fashion provides the strongest evidence of a 5-HT_4 receptor involvement. GR 113808 is a highly selective antagonist at 5-HT_4 receptors having no affinity for 5-HT_1, 5-HT_2 or 5-HT_3 receptors or other receptors at the concentration employed in the present study (Grossman *et al.*, 1993). As only one concentration of GR 113808 could be tested due to the finite number of tissues available, the existence of an equilibrium condition cannot be assumed. This non equilibrium condition probably accounts for the slightly lower potency of GR 113808 (apparent pK_B values 8.3 and 8.4 with 5-HT and 5-methoxytryptamine respectively) as compared to 9.2 in guinea-pig proximal colon, 9.5 in rat oesophagus (Grossman *et al.*, 1993), 8.8–9 in rat terminal ileum (Tuladhar *et al.*, 1994) and 8.8 in human atrium (Kaumann, 1993).

Whilst the 5-HT_4 receptor may play a major role in facilitating the peristaltic reflex in the marmoset ileum in response to exogenous agonist challenge, there is the possibility that the 5-HT_3 receptor is also involved in the facilitatory effect of higher concentrations of 5-HT. This is indicated in two ways. Firstly, by the ability of a higher concentration of 3 or 10 μM 2-methyl-5-HT but not 5-methoxytryptamine to facilitate peristalsis in tissues previously treated with high concentration of 10 μM 5-methoxytryptamine. 5-Methoxytryptamine causes selective desensitization of the 5-HT_4 receptor without affecting the 5-HT_3 receptor in the guinea-pig ileum (Craig *et al.*, 1990) and in the present experiments also selectively abolished the effects of 5-methoxytryptamine. Secondly, ondansetron, a highly selective 5-HT_3 receptor antagonist abolished the response to 2-methyl-5-HT. Thus the ability of 5-HT to produce the facilitatory effect on peristalsis appears to be first an action

mediated via the 5-HT_4 receptor, which is followed, with an increase in concentration of 5-HT, by an action on the 5-HT_3 receptors, an effect qualitatively similar to that observed in the guinea-pig ileum (Tuladhar *et al.*, 1995). However, differences in the potency of 5-HT to stimulate the 5-HT_3 and 5-HT_4 receptors make it unlikely that the 5-HT_3 receptors have a major role in facilitating the peristaltic reflex. The mechanism mediating the inhibitory effect of higher concentrations of 5-HT in the marmoset ileum is yet to be investigated.

Studies in the guinea-pig have indicated that 5-HT_4 receptor stimulation to facilitate gastrointestinal motility and peristalsis involves an increased cholinergic function (Craig & Clarke, 1990; Kilbinger & Wolf, 1992; Costall *et al.*, 1993). In the present study, the ability of a serosal application of a high concentration of acetylcholine to cause spasm of the marmoset ileum indicates an excitatory cholinergic input to the circular muscle. However, the inability of acetylcholine to stimulate the peristaltic reflex in the marmoset ileum clearly differed from the response observed in the guinea pig ileum (Costall *et al.*, 1993). This failure may reflect the lesser importance of the cholinergic system to peristalsis in the new world primate. However, it should not be ignored that the administration of acetylcholine via the lumen or *in vivo* via the vasculature might give a different result.

Thus, the present study indicates that the marmoset ileum can be used as a model to study segmentation and the peristaltic reflex, that exogenous 5-HT produces both facilitatory and inhibitory effects depending on the concentration of 5-HT employed and that the facilitatory effects are mediated via 5-HT_4 and 5-HT_3 receptors.

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