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Pharmacological characterization of the 5-hydroxytryptamine receptor mediating relaxation in the rat isolated ileum

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1 The aim of the present study was to investigate a 5-HT_4 receptor involvement in the mediation of a 5-HT-induced relaxation response in the rat isolated ileum *in vitro*.

2 Ileal segments were taken at regular intervals from the ileo-caecal junction to duodenum. 5-HT (1 μM) induced a relaxation or contraction response in segments taken from the terminal ileum: the relaxation decreased and finally disappeared as contractions dominated in the proximal tissues. The 5-HT-induced relaxations were enhanced in the terminal segments and the contractions attenuated in both terminal and proximal segments, in the presence of methysergide (1 μM) and atropine (0.1 μM).

3 In the presence of methysergide (1 μM) and atropine (0.1 μM), a cumulative addition of 5-HT (0.01-1 μM) induced a concentration-dependent relaxation in the terminal (1-20 cm from the ileo-ceacal junction) ileal segments which at higher concentrations of 5-HT (3-30 μM) reverted to contraction.

4 The rank order of potency of indole agonists in inducing a concentration-related relaxation response in tissues of the terminal ileum (pretreated with pargyline (100 μM) and in the presence of methysergide (1 or 100 μM) and atropine (0.1 μM)) was 5-hydroxytryptamine (6.97 ± 0.06), 5-methoxytryptamine (6.50 ± 0.07), α-methyl-5-hydroxytryptamine (5.53 ± 0.17), 5-carboxamidotryptamine (5.51 ± 0.12) and 2-methyl-5-hydroxytryptamine (<5), the pEC_{50} values (mean $\pm$ s.e.mean) being shown in parentheses.

5 Pretreatment of tissues with pargyline (100 μM) selectively enhanced the potency of 5-methoxytryptamine by a factor of 19 but failed to modify the potency of the other indole agonists.

6 The 5-HT_4 receptor antagonists, tropisetron, SDZ 205-557 and GR 113808 antagonized the relaxation response to 5-HT (in the presence of methysergide (1 or 10 μM) and atropine (0.1 μM)) with pK_B values (95% CL) of 6.09 (5.94-6.24), 7.0 (6.9-7.09) and 8.95 (8.81-9.1) respectively. Apparent pK_B values estimations for tropisetron (1 μM) and GR 113808 (10 nM) using the agonists 5-methoxytryptamine and 5-carboxamidotryptamine were 6.37 ± 0.31, 5.91 ± 0.38 and 8.83 ± 0.11, 8.82 ± 0.22 respectively.

7 Tropisetron (10 μM), SDZ 205-557 (3 μM) and GR 113808 (10-100 nM) caused an increase in basal tone of the rat terminal ileum when administered in the presence of methysergide and atropine.

8 The relaxation response to 5-HT in the rat terminal ileum was not antagonized by ritanserin (1 μM), ondansetron (1 μM) or N^{ω}-nitro-L-arginine methyl ester (100 μM) and with only a twofold dextral shift of the concentration-response curve by tetrodotoxin (1 μM).

9 It is concluded that the relaxant response to 5-HT in the terminal region of the ileum is mediated directly at the smooth muscle; a ranked indole agonist potency and selective antagonism by 5-HT_4 receptor antagonists tropisetron, SDZ 205-557 and GR 113808 indicate a 5-HT_4 receptor involvement in the relaxation response.

Keywords: 5-Hydroxytryptamine; 5-HT_4 receptor; rat ileum; relaxation; tropisetron; SDZ 205-557; GR 113808

Introduction

The 5-HT_4 receptor belongs to the seven transmembrane domain G protein coupled receptor superfamily, activation of which results in the stimulation of adenylyl cyclase and elevation of adenosine 3':5'-cyclic monophosphate (cyclicAMP) (Hoyer *et al.*, 1994). In the gut, activation of the 5-HT_4 receptor in *in vitro* experiments causes contraction of guinea-pig ileum (Craig & Clarke, 1990) and colon (Elswood *et al.*, 1991) by the release of acetylcholine from the enteric neurones. 5-HT_4 receptor stimulation also increases peristaltic reflex sensitivity in isolated intact preparations taken from the guinea-pig ileum (Craig & Clarke, 1991; Buchheit & Buhl, 1991; Costall *et al.*, 1993) and marmoset (Tuladhar *et al.*, 1996) and the facilitation of the peristaltic reflex may explain the prokinetic effect of benzamide derivatives which are agonists at the 5-HT_4 receptor (Dumuis *et al.*, 1989). Furthermore, 5-HT_4 receptor stimulation has been shown to increase gastric emptying in rats (Hedge *et al.*, 1995) and to be involved in the 5-hydroxytryptophan-induced defaecation and diarrhoea in mice (Banner *et al.*, 1993; Hedge *et al.*, 1994). In addition to enhancing gut motility, an increase in the secretion from the rat and human gastrointestinal mucosa (Bunce *et al.*, 1991; Borman & Burleigh, 1993; Burleigh & Borman, 1993) has also been shown to be mediated by the 5-HT_4 receptor. However, a physiological role of the 5-HT_4 receptor in gut motility has remained uncertain since 5-HT_4 receptor antagonists such as SB 204070 when administered alone fail to reduce faecal pellet output (Banner *et al.*, 1993) and the intestinal transit of radioactive microspheres administered into the duodenum of the conscious rat is not altered by the administration of either 5-HT_4 receptor agonists or antagonists (Clayton & Gale, 1996).

In addition to enhancing gut motility, stimulation of the 5-HT_4 receptor located on smooth muscle causes a relaxation of the rat oesophagus and terminal ileum (Reeves *et al.*, 1991; Baxter *et al.*, 1991; Tuladhar *et al.*, 1991), human intestinal smooth muscle (Kuemmerle *et al.*, 1995) and a decrease of spontaneous activity of the human colon (Tam *et al.*, 1993). A relaxation potential mediated via a 5-HT_4 receptor could potentially attenuate a contractile effect. The aim of the present

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study was to investigate the involvement of the 5-HT$_4$ receptor in the mediation of the relaxation response in the rat ileum. A preliminary account of some of these findings has been presented at meetings of The British Pharmacological Society, July 1991 (Tuladhar *et al.*, 1991) and December 1991 (Tuladhar *et al.*, 1992).

Methods

Animals and housing conditons

Female Hooded Lister rats (Bradford strain) weighing 200–300 g were housed in groups of 5–6 in a cage (56 cm × 38 cm × 18 cm) and allowed food and water *ad libitum*. The animals were maintained in a constant environment of 18–19°C on a 14 h/10 h light dark cycle.

Preparation of tissue

Animals were stunned by a blow to the head and killed by cervical dislocation. The abdomen was opened, a length of small intestine from a designated region was removed and transferred to a 50 ml organ bath containing Krebs-Henseleit solution (composition: NaCl 118, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25.0, glucose 10.0 mM) kept at 37°C and oxygenated with 95% O_2 and 5% CO_2. The ileum was removed from the bath for a minimum time necessary during further preparation of the tissue and kept oxygenated and warm at all times. The mesentery was cut away, segments of the intestine (2–3 cm in length) were removed and the intraluminal content was flushed out using a 5 ml syringe filled with Krebs-Henseleit solution. The tissues were then mounted in 10 ml organ baths containing Krebs-Henseleit solution kept at 37°C and oxygenated with 95% O_2 and 5% CO_2. Changes in tissue tension were measured isometrically using Grass Force Displacement transducers (FT03C, Grass Instrument Co., Mass., U.S.A.) and recorded on a multichannel Grass recorder. The tissues were washed 4–6 times and equilibrated further for at least 45 min with washout every 15 min. Tension was then slowly applied to the tissues to a maximum of 1 g: the tension was increased or decreased as necessary until a stable baseline tension of 0.6–1 g was obtained. This procedure almost always produced quiescent tissues with little (less than 100 mg in amplitude) or no spontaneous activity. If higher spontaneous activity was present then the tension was removed, the tissues washed 2–3 times and the tension slowly reapplied after 10–15 min to attain a slightly lower value. Tissues from any animal still exhibiting high spontaneous activity were discarded.

Construction of concentration-effect curves

Non cumulative concentration-contraction/relaxation response curves were established by increasing the concentration of 5-HT added to the organ baths at intervals of 10 min. Each concentration was left in contact for 1 min. Cumulative concentration-relaxation response curves to 5-HT and other agonists were obtained by addition of the agonists with a 2 min contact time. In the experiments examining the variation in the relaxation response to 5-HT in the different regions of the small intestine, segments from different regions were challenged with a single concentration of 1 μM 5-HT.

Experimental protocol for studying the effects of 5-HT receptor agonists and antagonists in the terminal ileum (1–20 cm from the ileo-ceacal junction)

Concentration-response curves were constructed by the cumulative addition of 5-HT or other agonists. The buffer routinely contained atropine (0.1 μM) and methysergide (1 μM) except in the experiments involving GR 113808 when the methysergide concentration in the bathing medium was 10 μM and in experiments involving α-methyl-5-HT when the bathing medium contained 100 μM methysergide. In experiments designed to determine the rank order of agonist potency, tissues were treated for 30 min with pargyline (100 μM) followed by washout before the application of tension. The antagonist drugs were added either to the reservoir itself or added before the application of tension and equilibrated for at least 30 min before construction of the concentration-response curves to the test agonist. When the effects of the antagonists were being determined on the baseline tension, the antagonists were added after a stable baseline tension was obtained (0.6–0.75 g). The comparisons were made using paired preparations and in most experiments with duplicate tissues from the same animal in each group. The relaxation response was expressed as the percentage of the maximum response in the response curve giving the greatest response.

Analysis of results

The ability of the agonists to relax the ileum was expressed both in absolute terms, as pEC_{50} values (relative to individual maxima) and in terms of their relative potency versus 5-HT. Potency relative to 5-HT was obtained in the experiments in which two concentration-effect curves were obtained from the tissues of the same animal and expressed as an EC_{50} ratio. pEC_{50} values were determined graphically. All results are expressed as arithmetic mean together with s.e.mean or 95% confidence limits except the EC_{50} ratios which are expressed as the geometric mean.

The antagonist dissociation constants were determined in two ways. Firstly, in the experiments employing multiple concentrations of the antagonists, pA_2 values were calculated by the method of Arunlakshana & Schild (1959) at 50% response. The slope of the resulting straight line was determined by linear regression. If the slope was not significantly different from 1 the pK_B value was estimated restricting the slope to 1. Secondly in the experiments where a single concentration of antagonist was used, an apparent pK_B value was calculated using the formula:

$$\text{apparent } pK_B = \log_{10}(\text{concentration} - \text{ratio} - 1) - \log_{10}(\text{antagonist concentration})$$

assuming a competitive interaction. The significance of differences between the values was determined at $P<0.05$ using Student's unpaired two-tailed t test.

Drugs

5-Hydroxytryptamine maleate, 5-methoxytryptamine hydrochloride, N^{ω}-nitro-L-arginine methyl ester hydrochloride, tetrodotoxin, pargyline hydrochloride, atropine sulphate (Sigma), ondansetron hydrochloride dihydrate, GR 113808 ([1-[2-methylsulphonyl)amino] ethyl]-4-piperidinyl]methyl-1-methyl-1H-indole-3-carboxylate) maleate (Glaxo), SDZ 205-557 (2-methoxy-4-amino-5-chloro-benzoic acid 2-(diethylamino)ethyl ester) and tropisetron hydrochloride (Sandoz), 2-methyl-5-hydroxytryptamine maleate, α-methyl-5-hydroxytryptamine maleate and 5-carboxamidotryptamine maleate (RBI) were dissolved in distilled water and diluted in Krebs-Henseleit solution. Methysergide hydrogen maleate (Sandoz) was dissolved by sonication. The drugs were added in a volume not exceeding 100 μl to a bath volume of 10 ml.

Results

Effect of a non-cumulative addition of 5-HT

In the initial studies using tissues taken from the terminal ileum (taken 1–20 cm from the ileo-ceacal junction), a non-cumulative addition of 5-HT produced small relaxations up to

a concentration of 0.3 μM of 5-HT. Addition of higher concentrations of 5-HT (1–10 μM) produced only contractions which were accompanied by an increase in the spontaneous activity (Figure 1a). The addition of methysergide (1 μM) and atropine (0.1 μM) in the Krebs-Henseleit solution enhanced the relaxation and attenuated the contractile response. The maximum relaxation to 5-HT (217±41 mg, $n=5$) was obtained at 1 μM in the presence of methysergide and atropine (Figure 1b). The pEC_{50}±s.e.mean value for the relaxation response to 5-HT was 7.04±0.14. At higher concentrations of 5-HT (10–30 μM), there was a reduction in the relaxation response and the development of contraction.

Variations in the relaxation response to 5-HT (1 μM) in different regions of the rat small intestine

In the ileal segments taken from the terminal portion of the intestine (<20 cm from ileo-caecal junction), tissues taken from two out of four animals tested showed clear relaxations to 5-HT (1 μM). The terminal ileal segments from the remaining two animals exhibited contractions of small magnitude with the tissues from one of these animals failing to show any relaxation. All the segments of ileum in all animals derived from the portion proximal to 20 cm distance from the ileo-caecal junction produced only contractions (Figure 2a). The relaxation responses to 5-HT (1 μM) in the segments obtained from the terminal part of the ileum were enhanced and consistent in the presence of methysergide (1 μM) and atropine (0.1 μM), with no contractions observed in any tissue obtained from the terminal part of the ileum (Figure 2b). In the presence of methysergide and atropine, the contractile responses were also greatly reduced in tissues from the proximal parts of the ileum.

Effect of a cumulative addition of 5-HT

The cumulative addition of 5-HT allowed a much more rapid study of 5-HT concentration-relaxation response curves than using non-cumulative additions. In tissues taken from the terminal ileum, consistent and concentration-dependent relaxations were observed following the cumulative addition of 5-HT (0.01–1 μM) in the presence of methysergide (1 μM) and atropine (0.1 μM) but not in their absence. With a cumulative addition of higher concentrations of 5-HT (3–30 μM) no further relaxations were observed and indeed decreased and even reverted to contraction in some tissues. The 5-HT-induced relaxation had no effect on the spontaneous activity (if present) of the tissue. The contraction was usually accompanied by a high degree of spontaneous activity which persisted in most tissue even after washing out of 5-HT. This made the construction of a second concentration-response curve on the same tissue difficult and hence subsequent comparisons were made with paired preparations. A typical relaxation response to 5-HT and the concentration-relaxation response curves of four tissues from the terminal ileum is shown in Figure 3. Such tissues were taken: (a) nearest to the ileo-caecal junction and (b) adjacent to the first, (c) second and (d) third tissues and their response was indistinguishable. Hence such tissues were subsequently used to assess and compare agonist and antagonist potencies. An inrease in the methysergide concentration to 10 or 100 μM or atropine to 1 μM had no effect on the concentration-relaxation response curves to 5-HT, data not shown.

The response of the rat terminal ileum to 5-HT receptor agonists

In the tissues pretreated with pargyline, cumulative concentration-relaxation response curves to a range of indole agonists gave a rank order of agonist potency 5-HT > 5-methoxytryptamine > α-methyl-5-HT ≥ 5-carboxamidotryptamine > 2-methyl-5-HT (Figure 4, Table 1). The pretreatment of the tissues with pargyline was essential as it was shown to shift the response curve of 5-methoxytryptamine selectively to the right by approximately 19 fold (pEC_{50} values with and without pretreatment with pargyline 6.41±0.08 and 5.12±0.10 respectively, $n=6$). The pEC_{50} values obtained in the absence of pargyline (5-HT (6.79±0.09, $n=5$), 5-carboxamidotryptamine (5.72±0.08, $n=9$), α-methyl-5-HT (5.59±0.08, $n=6$) and 2-methyl-5-HT (<5, $n=5$)) were not changed significantly by the pretreatment of the tissues with pargyline but the pargyline treatment decreased the maximum response to 5-HT by 32% (data not shown). The construction of a relaxation-response

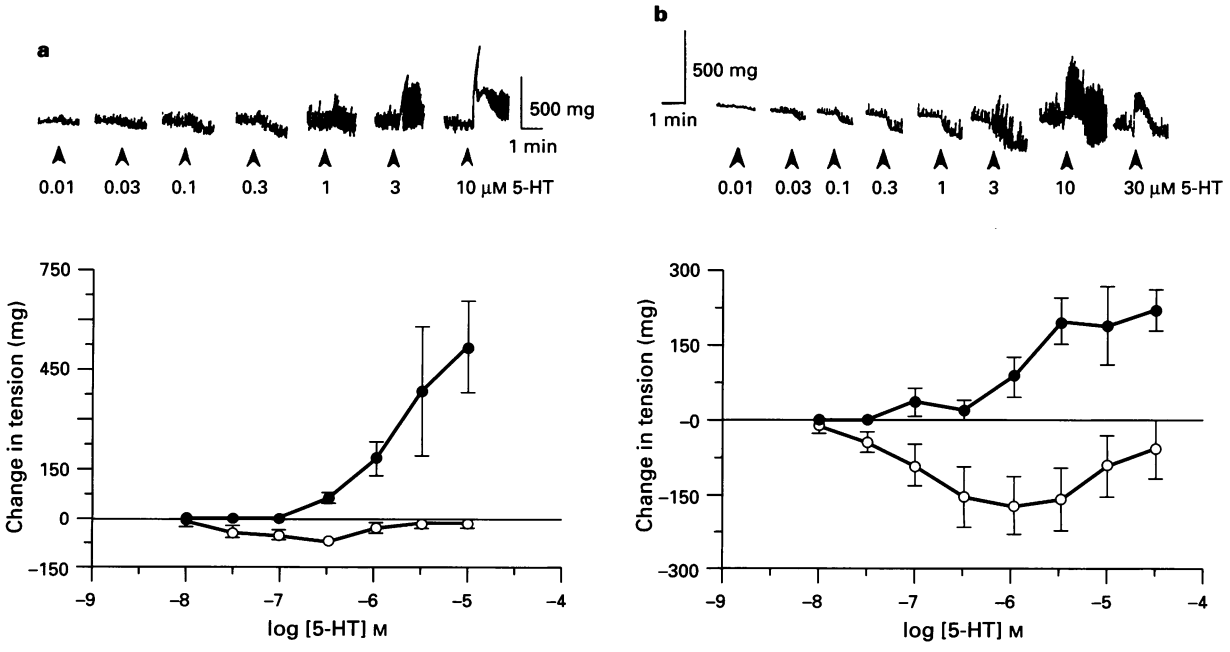

Figure 1 Representative traces and concentration-contraction (●) and-relaxation (○) response curves to 5-HT in the rat terminal ileum (taken 1–20 cm from the ileo-caecal junction) to the non-cumulative addition of 5-HT at 10 min intervals (a) in absence of any antagonists and (b) in the presence of methysergide (1 μM) and atropine. (0.1 μM). Values shown in the concentration-response curves are the means with s.e.mean in tissues taken from 3–5 animals. Arrows in the tracing indicate the addition of 5-HT.

curve to a high concentration of α-methyl-5-HT required the use of 100 μM methysergide. This concentration of methysergide had no effect on the concentration-relaxation response induced by 5-HT but blocked a slow loss of tone previously seen at or above 1 μM α-methyl-5-HT when used in the presence of 1 μM methysergide. The latter effect was always accompanied by abolition of spontaneous activity (if present) in the tissue and eventually caused a complete loss of the tissue tone. In some tissues there was an apparent biphasic relaxation response to 5-carboxamidotryptamine. However, the initial relaxation (at concentrations below 0.3 μM) observed in these tissues was very small and difficult to reproduce in all experiments. The response curve to 5-carboxamidotryptamine at concentrations higher than 0.3 μM (1–10 μM) was, however, essentially parallel to that of 5-HT.

The effect of tropisetron, SDZ 205-557 and GR 113808 on the relaxation response induced by 5-HT receptor agonists

Tropisetron (1–10 μM), SDZ 205-557 (0.3–3 μM) and GR 113808 (0.01–0.1 μM) caused parallel dextral shifts of the concentration-response curve to 5-HT (Figure 5). A lower concentration of tropisetron (0.1 μM) had no effect, data not shown. Schild analysis revealed slopes not significantly different from unity. The pA_2 values taken as the intercept on the x-axis on the Schild plot, slopes and the pK_B values estimated constraining the slope to unity are presented in Table 2.

Both tropisetron (1 μM) and GR 113808 (0.01 μM) aslo caused an essentially parallel dextral shift of the concentration-response curves to 5-methoxytryptamine and 5-carboxamidotryptamine. The apparent pK_B estimates are presented in Table 3.

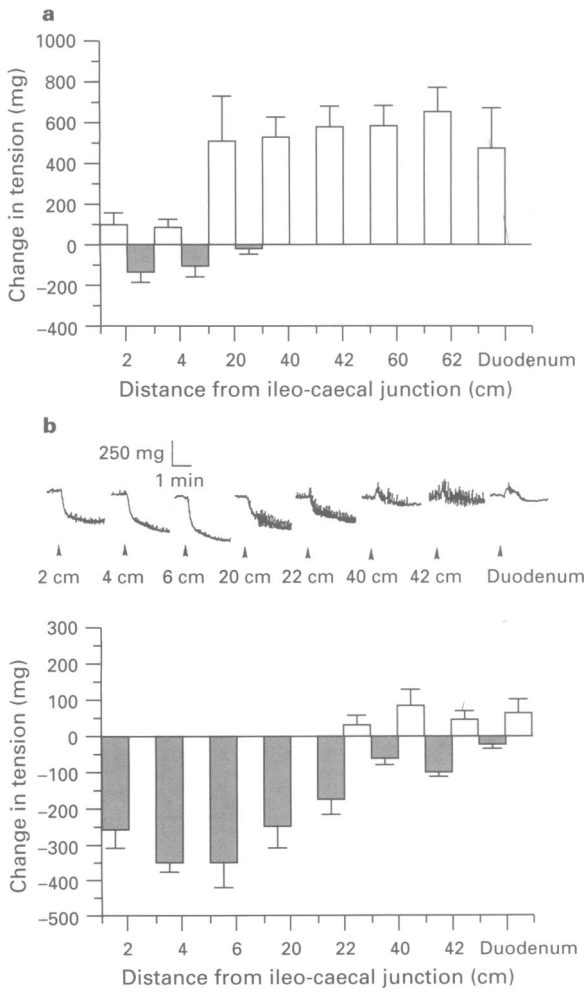

Figure 2 The contractile (open columns) and relaxant (stippled columns) response of different regions of the rat ileum and duodenum to 5-HT (1 μM) in (a) absence of any antagonist and (b) in the presence of methysergide (1 μM) and atropine (0.1 μM) in the bathing medium. Values shown in the graphs are mean with s.e.mean in tissues taken from 4–6 animals. An example tracing of the responses to 5-HT in the presence of methysergide and atropine is also presented. Arrows in the tracing indicate the addition of 5-HT (1 μM).

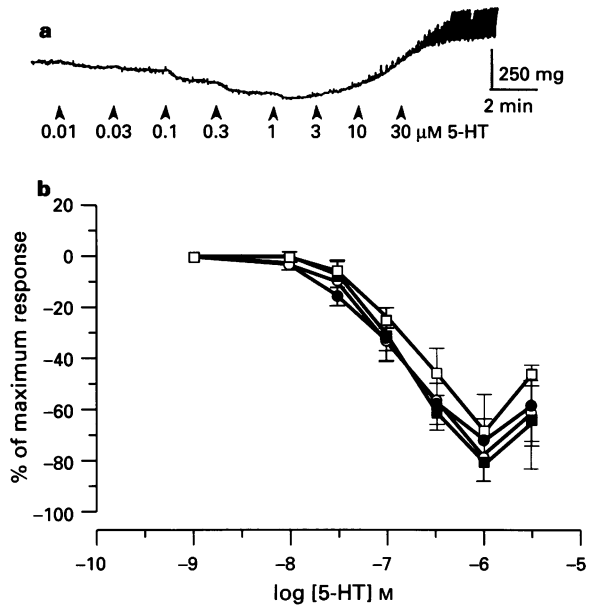

Figure 3 (a) An example trace of the changes in baseline tension on cumulative addition of 5-HT at 2 min intervals in the rat terminal ileum in the presence of methysergide (1 μM) and atropine (0.1 μM). Arrows indicate the addition of 5-HT. (b) Comparison of the relaxation-response curves to 5-HT in four terminal tissues: (○) nearest to the ileo-ceacal junction; (●) adjacent to the first tissue; (□) adjacent to the second tissue and (■) adjacent to the third tissue. Values shown are the means with s.e.mean in tissues taken from 6 animals. Results are presented as a percentage of the maximum response in the tissue giving the largest relaxation.

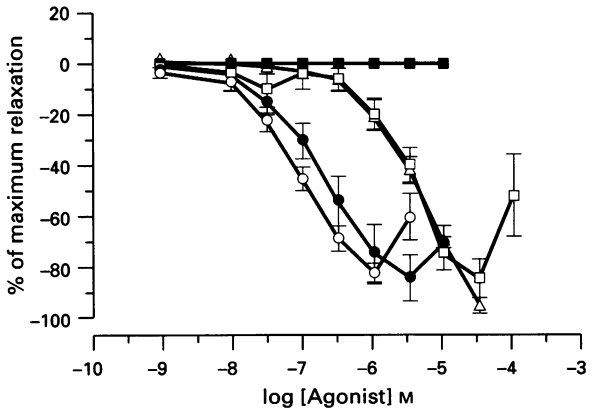

Figure 4 Concentration-relaxation response curves to 5-hydroxytryptamine and other indole 5-HT receptor agonists in the rat terminal ileum pretreated with pargyline (100 μM for 30 min). (a) Relaxation response curves to 5-HT (○, $n=15$), 5-methoxytryptamine (●, $n=5$), 5-carboxamidotryptamine (□, $n=5$), 2-methyl-5-HT (■, $n=3$) and α-methyl-5-HT (△, $n=5$). Buffer routinely contained atropine (0.1 μM) and methysergide (1 μM) except in the experiment with α-methyl-5-HT when it contained atropine (0.1 μM) and methysergide (100 μM). Values shown are the means with s.e.mean from a number of animals indicated by *n*.

The effect of tropisetron, SDZ 205-557 and GR 113808 on quiescent tissues

The three 5-HT_4 receptor antagonists, tropisetron (10 μM), SDZ 205-557 (3 μM) and GR 113808 (0.01–0.1 μM) when administered alone increased the tone of the quiescent tissues (by approximately 100 to 200 mg, $n=6$, data not shown). With all of these antagonists, there was a time lag of about 3 min before the increase in the tone became evident. GR 113808 (0.3 μM) could also reverse the relaxation due to 5-HT (0.3 μM). The stable baseline obtained after the reversal of the relaxant effect of 5-HT by GR 113808 was also above (231 ± 6 mg, $n=2$) the initial baseline tension.

The effect of ondansetron, ritanserin, tetrodotoxin and N^{ω}-nitro-L-arginine methyl ester (L-NAME) on the relaxation response to 5-HT

The inclusion of ondansetron (1 μM), tetrodotoxin (0.1 μM), ritanserin (0.1 μM) or L-NAME (30 or 100 μM) in the bathing medium had no effect on the concentration-relaxation response curve to 5-HT. The fade of the relaxation at higher

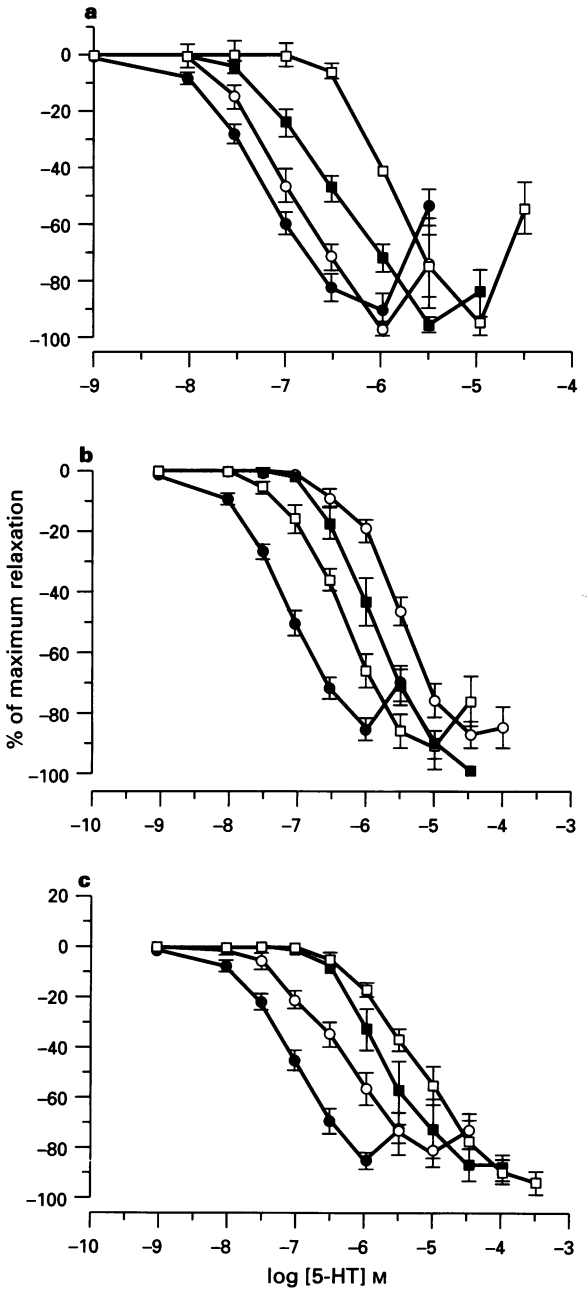

Figure 5 Antagonism of the relaxation response to 5-HT in the rat terminal ileum by tropisetron, SDZ 205-557 and GR 113808. Concentration-relaxation response curves to 5-HT (a) in the absence (●, $n=18$) and presence of 1 (○, $n=7$), 3 (■, $n=6$) and 10 μM (□, $n=6$) tropisetron; (b) in the absence (●, $n=18$) and presence of 0.3 (□, $n=6$), 1 (■, $n=6$) and 3 μM (○, $n=6$) SDZ 205-557; (c) in the absence (●, $n=18$) and presence of 0.01 (○, $n=6$), 0.03 (■, $n=6$) and 0.1 μM (□, $n=6$) GR 113808. Buffer routinely contained atropine (0.1 μM) and methysergide (1 μM) except in the experiment with GR 113808 when it contained atropine (0.1 μM) and methysergide (10 μM). Values shown are the means with s.e.mean from a number of animals indicated by n.

Table 1 Potency of the 5-HT receptor agonists in causing a relaxation response in the rat terminal ileum pretreated with 100 μM pargyline for 30 min followed by washout

Agonists	*pEC_{50} (±s.e.mean)*	n	*EC_{50} ratio*	*E_{max}*	n
5-Hydroxytryptamine	6.97±0.06	20		100	
5-Methoxytryptamine	6.50±0.07	11	2	105	5
α-Methyl-5-HT*	5.53±0.17*	5	32	111**	5
5-Carboxamido tryptamine	5.51±0.12	5	30	107	5
2-Methyl-5-HT	<5	3			

The bathing medium routinely contained methysergide (1 μM) and atropine (0.1 μM). pEC_{50} values shown are the collective data from the maximum number of animals used where the EC_{50} ratio and E_{max} values for agonists are calculated by comparison of the response with the effect of 5-HT in tissues from the same animal.
*In the presence of 100 μM methysergide; **the response to 30 μM α-methyl-5-HT, the highest concentration employed.

Table 2 Potency of the 5-HT_4 receptor antagonists in antagonizing the 5-HT induced relaxation in the rat terminal ileum

Antagonists	*pA_2**	*Slope (95% CL)*	*pK_B**(95% CL)*
Tropisetron	6.03	1.13(0.85–1.41)	6.09(5.94–6.24)
SDZ 205-557	7.04	0.96(0.54–1.37)	7.0 (6.9–7.09)
GR 113808	8.61	1.31(0.90–1.72)	8.95(8.81–9.10)

The buffer routinely contained atropine (0.1 μM) along with either 1 μM (in experiments using tropisetron or SDZ 205-557) or 10 μM methysergide (in the experiments using GR 113808). *Slope not restricted to 1; **Calculated by restricting the slope to unity.

Table 3 Apparent pK_B estimations with tropisetron and GR113808 using a single concentration of the antagonists

Agonists	*Antagonists (concentration)*	*Apparent pK_B (±s.e.mean)*	n
5-Methoxytryptamine	Tropisetron (1 μM)	6.37±0.31	4
5-Carboxamido tryptamine	Tropisetron (1 μM)	5.91±0.38	4
5-Methoxytryptamine	GR 113808 (10 nM)	8.83±0.11	6
5-Carboxamido tryptamine	GR 113808 (10 nM)	8.82±0.22	4

The buffer routinely contained atropine (0.1 μM) along with either 1 μM (in experiments using tropisetron) or 10 μM methysergide (in the experiments using GR 113808).

Table 4 The potency of 5-HT in causing relaxant response in the rat terminal ileum in the presence of 5-HT receptor antagonists, tetrodotoxin and L-NAME

Antagonists	Concentration tested	Relaxant responses to 5-HT ($pEC_{50} \pm$ s.e.mean) Without antagonist	With antagonist	n
Ondansetron	1 μM	7.27±0.14	7.21±0.05	6
Ritanserin	0.1 μM	7.27±0.14	7.05±0.12*	5
Tetrodotoxin	0.1 μM	7.34±0.11	7.30±0.08	6
Tetrodotoxin	1 μM	7.56±0.08	7.19±0.08**	6
L-NAME	30 μM	6.99±0.11	6.91±0.11	4
L-NAME	100 μM	6.99±0.11	6.94±0.07	4

Methysergide (1 μM) and atropine (0.1 μM) were routinely included in the bathing medium unless otherwise mentioned. *In the absence of methysergide and atropine in the bathing medium; **difference statistically significant: Students *t* test, unpaired two tail.

concentrations of 5-HT was also not affected by the presence of the above antagonists. An approximate two fold shift of the concentration-response curve to 5-HT was, however, seen in the presence of 1 μM tetrodotoxin. The pEC_{50} values for 5-HT in the presence and absence of the above antagonists are presented in Table 4.

Discussion

The dominant effect of submicromolar concentrations of 5-HT in the rat terminal ileum (defined as tissues taken 1–20 cm from the ileo-caecal junction) was a relaxation response but concentrations higher than 1 μM induced contraction. Regionally a concentration of 1 μM 5-HT induced variable contraction or relaxation when tissues were taken 20 cm or more from the ileo-caecal junction, but the contractile response began to dominate until this reflected the sole component in the more proximal tissues. The appearance of the contraction is probably related to two distinct factors. Firstly, a direct effect of 5-HT acting at contractile-inducing 5-HT receptors sensitive to methysergide and also those receptors mediating contraction via a cholinergic (atropine sensitive) mechanism; using 1 μM 5-HT a combined treatment with methysergide plus atropine abolished the contraction response seen in some tissues from the terminal ileum and attenuated contractions in more proximal tisses. Secondly it is also possible that a desensitization of the 5-HT receptor mediating the relaxation response was induced by higher concentrations of 5-HT in the terminal ileum. Subsequent studies focused on a characterization of the 5-HT receptor mediating the relaxation response in the terminal ileum.

The routine inclusion of methysergide (up to 100 μM) in the buffer medium, excluded the relaxant response in the rat terminal ileum being mediated by the 5-HT_{1E} and 5-HT_{1F} receptors (Amlaiky *et al.*, 1992; Adham *et al.*, 1993); the $5\text{-HT}_{2a/b/c}$ receptors (Hoyer *et al.*, 1994; Baxter *et al.*, 1994) or the $5\text{-HT}_{5\alpha}$, $5\text{-HT}_{5\beta}$, and 5-HT_7 receptors (Erlander *et al.*, 1993; Ruat *et al.*, 1993). The lower potency of 5-carboxamidotryptamine as compared to 5-HT in causing a relaxation response also indicates that the response is unlikely to be mediated by the 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D} or $5\text{-HT}_{1\text{-like}}$ receptors (Bradley *et al.*, 1986; Hoyer *et al.*, 1994) or the 5-HT_6 receptor (Monsma *et al.*, 1993). The involvement of the putative 5-HT_{1P} receptor described by Mawe *et al.* (1986) can also be discounted since tropisetron (even at higher concentrations) and 5-methoxytryptamine were reported to be inactive (Branchek *et al.*, 1988). The involvement of the 5-HT_3 receptor in the relaxation response to 5-HT is also unlikely, due to the failure of ondansetron (1 μM) and a lower concentration of tropisetron (0.1 μM) to antagonize the response. These concentrations of ondansetron and tropisetron are at least 10 and 100 times respectively their reported affinity at the 5-HT_3 receptors (Humphrey *et al.*, 1993).

Direct evidence for the involvement of the 5-HT_4 receptor in the relaxation response to 5-HT in the rat terminal ileum comes from the antagonism of the relaxation response in a surmountable manner with all three 5-HT_4 receptor antagonists tested, namely tropisetron, SDZ 205-557 and GR 113808. In the present study the pK_B value calculated for tropisetron with the slope restricted to 1 for 5-HT is 6.09 (95% CL 5.94-6.24), which is comparable to the potencies of tropisetron in antagonizing 5-HT_4 receptors in the mouse colliculi neurones (pK_i value 6.15±0.14) (Dumuis *et al.*, 1988a), rat oesophagus tunica muscularis mucosa (pA_2 6–6.7) (Baxter *et al.*, 1991) and guinea-pig ileum (pA_2 6.3–6.6) (Craig & Clarke, 1990). The apparent pK_B values obtained for tropisetron using 5-methoxytryptamine and 5-carboxamidotryptamine (6.37 and 5.91 respectively) were similar to the values obtained with 5-HT. This agonist-independence of the pK_B values further supports the single site of agonist action with these compounds.

Tropisetron is not a selective antagonist of 5-HT_4 receptors. It has a higher potency at 5-HT_3 receptors (Richardson *et al.*, 1985) and at high concentrations it can affect potassium, sodium and calcium currents (Scholtysik *et al.*, 1988). However, there is no evidence of such nonselective effects of tropisetron hampering the characterization of the 5-HT_4 receptor in the present study. Furthermore the more selective 5-HT_4 receptor antagonists, SDZ 205-557 (Buchheit *et al.*, 1992) and GR 113808 (Grossman *et al.*, 1993) shifted the concentration-relaxation response curves to 5-HT to the right in a parallel and surmountable manner. The pK_B value of 7 (95% CL 6.9–7.09) obtained for SDZ 205-557 in the rat ileum is comparable with the antagonist potencies of SDZ 205-557 in other 5-HT_4 systems, the quiescent and electrically stimulated guinea-pig ileum (pA_2 7.4 and 7.3 respectively) (Buchheit *et al.*, 1992), rat tunica muscularis mucosa (pA_2 7.3) (Eglen *et al.*, 1993) and guinea-pig hippocampal adenylyl cyclase (pA_2 7.5) (Eglen *et al.*, 1993). The pK_B value obtained for GR 113808 was 8.95 (95% CL 8.81–9.10) and is in agreement with the previously obtained value for the 5-HT_4 system in the human atrial appendages (pK_B value 8.8) (Kaumann, 1993), guinea-pig proximal colon (pA_2 9.2) and rat oesophagus (pA_2 9.5). Agonist-independence of the pK_B values was again demonstrated with GR 113808 using 5-methoxytryptamine (8.83) and 5-carboxamidotryptamine (8.82).

Support for a 5-HT_4 receptor involvement in the relaxation response to 5-HT in the rat terminal ileum also comes from the rank order of potency of the indole agonists obtained when metabolic interference due to monoamine oxidase enzymes was prevented. The rank order thus obtained: 5-HT > 5-methoxytryptamine > α-methyl-5-HT ≥ 5-carboxamidotryptamine > 2-methyl-5-HT is the same as the rank of potency for the indole 5-HT receptor agonists to stimulate the 5-HT_4 receptor system in the longitudinal muscle myenteric plexus of the guinea-pig ileum (Craig & Clarke, 1990), the rat oesophagus muscularis mucosa (Baxter *et al.*, 1991) and the mouse colliculi neurones (Dumuis *et al.*, 1988b). The selective breakdown of 5-methoxytryptamine but not other indole agonists may explain some of the discrepant results on the rank order of agonist potencies obtained in different tissues. Thus in the rat oesophagus Reeves *et al.* (1991) found 5-methoxytryptamine to be less potent than 5-carboxamidotryptamine whereas using the tunica muscularis mucosa treated with pargyline, other investigators have found 5-methoxytryptamine to be nearly equipotent to 5-HT and more potent than 5-carboxamidotryptamine (Baxter *et al.*, 1991; Eglen *et al.*, 1992). It is interesting to note that such lower potency estimates of 5-methoxytryptamine are observed in the gastrointestinal tissues obtained from the rat (Vane, 1959; Reeves *et al.*, 1991; Baxter *et al.*, 1994) but not the guinea-pig (Craig & Clarke, 1990; Elswood *et al.*, 1991; Eglen *et al.*, 1992; Costall *et al.*, 1993). It should be noted that the use of α-methyl-5-HT required a higher concentration of methy-

sergide. The slow loss of tone accompanied by abolition of spontaneous activity observed with α-methyl-5-HT in the presence of a lower concentration of 1 μM methysergide in the buffer is probably a 5-HT$_2$ receptor-mediated effect; it is observed only with α-methyl-5-HT which is known to have higher affinity at 5-HT$_2$ receptors (Hoyer *et al.*, 1994) and the effect was blocked by a higher concentration of methysergide (100 μM).

The relaxant response to 5-HT in the rat terminal ileum was not abolished by tetrodotoxin at either 0.1 or 1 μM, concentrations sufficient to block the action potential in the nerve cells (Gershon, 1967). This indicates that the receptor mediating the relaxation response in the rat terminal ileum is probably located on the smooth muscle cells. The significance of a small and an approximate two fold shift of the 5-HT concentration-response curve to the right with 1 μM tetrodotoxin is uncertain. Nevertheless, this may have resulted from some antagonism of 5-HT$_4$ receptor function since a slight shift in the response curves has also been noted in other 5-HT$_4$ systems located on smooth muscle such as human colonic circular muscle (Tam *et al.*, 1993). In the present study the exact ileal muscular layer responsible for mediating the observed relaxation is not known. Although the tissues were mounted in the longitudinal axis, the relaxation cannot be simply deduced to be due to relaxation of the longitudinal muscle as even the contraction of the circular muscle may also passively elongate the tissues (Wood & Perkins, 1970), giving an apparent effect of relaxation. Furthermore, the possibility of an involvement of other muscle layers such as the muscularis mucosa cannot be ignored. The inactivity of L-NAME indicates the non-involvement of nitric oxide in the 5-HT-induced relaxations.

The occurrence of the 5-HT$_4$ receptor-mediated relaxant response to 5-HT varied in different regions of the rat small intestine, with a clear and consistent relaxation to 5-HT occurring only in the segments obtained from the terminal region (approximately 20 cm) of the intestine. This variation in the relaxation response to 5-HT may reflect the variation in 5-HT$_4$ receptor density or an increasing dominance of the contracile function. It would be of interest to assess 5-HT$_4$ receptor density throughout the GI tract using an autoradiographic study. Such studies would also further an understanding of the exact location of the receptor in the rat ileum.

The ability of endogenous 5-HT to influence the 5-HT$_4$ receptor was shown in two ways. Firstly, the increase in the basal tone of the tissues due to the administration of the 5-HT$_4$ receptor antagonists, tropisetron, SDZ 205-557 and GR 113808 alone is hypothesized to be due to the antagonism of an endogenous activation of the 5-HT$_4$ receptor mediating a relaxant tone. The comparable maximum increase in tone irrespective of the antagonists used makes it unlikely that the increase in the tone is a specific property of the individual antagonists. Secondly, desensitization of the 5-HT$_4$ receptor using higher concentrations of exogenous 5-HT causes an increase in tone. The ability of endogenous 5-HT to stimulate 5-HT$_4$ receptors has previously been shown to exist in the rat tunica muscularis mucosa (Waikar *et al.*, 1993). The endogenous 5-HT concentration at the receptor site after pargyline treatment might be increased after prevention of metabolism by monoamine oxidase enzymes. This might have resulted in partial desensitization resulting in the lower maximum response seen with 5-HT after the pargyline treatment.

In summary, the present study has demonstrated a role for the 5-HT$_4$ receptor in mediating a relaxation response in the rat ileum. The relaxation is consistently recorded in the terminal ileum whereas in other areas a non-5-HT$_4$ receptor mediated contractile response is dominant. A balanced relaxation and contraction potential of 5-HT may contribute importantly to patterns of intestinal motility, physiological and perhaps pathological functions of 5-HT and the design of agents to treat disorders of motility.

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