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Do 5-HT_4 Receptors Mediate the Intestinal Secretory Response to 5-HT in Rat In-vivo?

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Abstract

The involvement of the recently characterized 5-HT_4 receptor in the actions of 5-hydroxytryptamine (5-HT) on jejunal, ileal and colonic electrogenic ion secretion was investigated in the rat in-vivo.

5-HT and the 5-HT_1-, 5-HT_2- and 5-HT_4-receptor agonist 5-methoxytryptamine (5-MeOT), induced a rise in transintestinal PD in all regions of the gut. However, the 5-HT_4-receptor agonists renzapride and cisapride had no effect. Furthermore, the 5-HT_4-receptor antagonists SDZ 205-557 (2-diethylaminoethyl-[2-methoxy-4-amino-5-chloro] benzoate), tropisetron and SB 204070 ([1-butyl-4-piperidinylmethyl]-8-amino-7-chloro-1,4-benzodioxan-5-carboxylate hydrochloride) did not affect the secretory response to either 5-HT or 5-MeOT in the jejunum, but did cause a small inhibition in the ileum and colon.

It is concluded that 5-HT_4 receptors do not make a contribution to the electrically monitored 5-HT intestinal secretory response in the rat jejunum in-vivo, but may play a small role in the ileum and colon.

5-Hydroxytryptamine (5-HT) can induce a net ion and fluid secretion in the intestine of a variety of different species (Cooke 1987). In-vitro experiments in the rat have shown that this secretion is due to a simultaneous inhibition of neutral NaCl absorption and stimulation of electrogenic Cl^- secretion (Hardcastle et al 1981). However, in different regions of the gut and different species, HCO_3^- ion secretion may also be involved (Urquhart et al 1988; Sundaram et al 1991).

The receptors mediating this secretion are as yet unclear and appear to vary with the species and intestinal region investigated. Evidence for 5-HT_2 receptor involvement has been found in the rat jejunum (Beubler & Horina 1990). 5-HT_3 receptors have also been shown to contribute to the response in the rat jejunum and proximal colon (Beubler & Horina 1990; Hardcastle et al 1992) and guinea-pig ileum (Baird & Cuthbert 1987). Recently, a further subgroup of 5-HT receptors, the 5-HT_4 receptor, has been characterized. 5-HT_4 receptor involvement in motility is well established (Tonini et al 1991), but its role in secretion is as yet unclear. 5-HT_4 receptor contribution has been implicated in-vitro in both the rat colonic response (Bunce et al 1991) and the maintained guinea-pig ileal response to 5-HT (Scott et al 1992) by excluding the involvement of 5-HT_1, 5-HT_2 and 5-HT_3 receptors with the use of selective antagonists at these receptors.

More recently, selective 5-HT_4-receptor antagonists have been used to demonstrate an involvement of this receptor in the transient rise in short-circuit current (SCC) in-vitro in guinea-pig ileum (Leung et al 1993), and in the human jejunum and ileum (Budhoo & Kellum 1993; Burleigh & Borman 1993) in response to stimulation by 5-HT.

The aim of this study was to clarify the role of 5-HT_4 receptors in mediating the secretory response to 5-HT in the rat in-vivo, using a variety of 5-HT receptor agonists and antagonists.

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Materials and Methods

Measurement of transintestinal electrical activity and cardiovascular function

Male Wistar rats, 230–250 g, from the Sheffield Field Laboratories, with free access to food and water, were anaesthetized by intraperitoneal injection of 70 mg kg^{-1} sodium pentobarbitone. Following tracheotomy, 5-cm segments of proximal jejunum, distal ileum and proximal colon were isolated by tying off at the distal end and inserting a cannula into the proximal end. The contents were washed out and the loops filled with warm 154 mM NaCl. The potential difference (PD) across each loop was measured between a salt bridge electrode in contact with the luminal fluid, and a common reference electrode in contact with the peritoneal fluid by means of a wick electrode. Each pair of electrodes was connected via calomel half-cells to a differential input electrometer. Blood pressure was measured via the femoral artery using a Druck pressure transducer (type 3389). Heart rate was calculated from the pulse pressure by a Lectromed rate meter (model 5250). Intestinal PDs, blood pressure and heart rate were all recorded on computer using CED Chart software. 5-HT-receptor agonists and antagonists were administered intravenously via the femoral vein in 0·1 mL and washed in with 0·2 mL saline.

Non-cumulative dose-response curves to agonists at 5-HT_4 receptors (5-hydroxytryptamine, 5-methoxytryptamine, renzapride and cisapride) were constructed using ascending doses before and after the administration of each of two doses of tropisetron, SDZ 205-557 (5-HT_4-receptor antagonists) or granisetron (a selective 5-HT_3-receptor antagonist). In the case of the 5-HT_4-receptor antagonist SB 204070, 5-HT dose-response curves were constructed before and

after a dose of 15 nmol kg^{-1}. As renzapride and cisapride were without significant effect on the intestinal PD, their effects after administration of antagonists were not investigated.

Expression of Results

Data were analysed using CED Spike2 software, and Student's paired or unpaired *t*-test as appropriate was used for statistical analysis. The effects of agonists were determined by subtraction of the basal PD just before administration from the maximum PD recorded. All data are expressed as the arithmetic mean ± s.e.m. ED50 values were obtained directly from dose-response curves.

Chemicals

Acetylcholine chloride (ACh), 5-hydroxytryptamine creatinine sulphate (5-HT) and 5-methoxytryptamine hydrochloride (5-MeOT) were obtained from Sigma Chemical Co., Poole, UK. Granisetron, tropisetron (ICS 205 930), SDZ 205-557 (2-diethylaminoethyl-[2-methoxy-4-amino-5-chloro] benzoate), renzapride and SB 204070 ([1-butyl-4-piperidinylmethyl]-8-amino-7-chloro-1,4-benzodioxan-5-carboxylate hydrochloride) were kindly donated by SmithKline Beecham Pharmaceuticals, Harlow, UK. Cisapride was kindly donated by Janssen Pharmaceutica, Beerse, Belgium.

All the drugs were dissolved in 0·9% NaCl (saline), except tropisetron and SDZ 205-557 which were dissolved in saline and 1 M HCl in a ratio of 4 : 1. This vehicle had no effect on cardiovascular function or transintestinal electrical activity. As SB 204070 may adhere to plastic it was administered using a glass syringe and the shortest possible cannula in the femoral vein.

Results

Effects of agonists

Basal PDs were 6·2 ± 0·3 mV in the jejunum, 4·0 ± 0·4 mV in the ileum and 14·0 ± 0·3 mV in the colon, the serosa being positive (n = 18 in each case).

5-HT induced a dose-dependent rise in transmural PD in the jejunum, ileum and colon (Fig. 1). The maximum increases in PD (PD_{max}) were 4·7 ± 0·3, 3·7 ± 0·5 and 7·0 ± 0·6 mV, respectively and the ED50 values were 20 ± 2, 35 ± 4 and 47 ± 8 nmol kg^{-1}, respectively (n = 6).

5-MeOT, an agonist at 5-HT_1, 5-HT_2 and 5-HT_4 receptors (Fozard 1990; Bockaert et al 1992) also induced a rise in PD in the jejunum, ileum and colon (Fig. 1). However, it was significantly less effective than 5-HT (Table 1). In the colon the PD_{max} for 5-MeOT was not reached as the fall in diastolic blood pressure prevented the administration of higher doses. The prokinetic benzamides, renzapride and cisapride, with 5-HT_4-receptor agonist properties (Dumuis et al 1989) had no effect on transmural PD in any of the intestinal regions examined (Fig. 1).

Intravenous administration of 5-HT induces a triphasic cardiovascular response (Fig. 2), with each phase being dose-dependent and mediated by activation of a different 5-HT receptor (Kalkman et al 1984). The first phase is a rapid and transient hypotension and bradycardia mediated by 5-HT_3 receptors on vagal afferents of the heart, known as the Bezold-Jarisch reflex. The second phase is a transient hypertension mediated by 5-HT_{2A}-like receptors (i.e. ketanserin-sensitive), followed by the third phase, a prolonged hypotension mediated by 5-HT_1-like receptors, both presumably on the vascular smooth muscle.

The maximum cardiovascular responses induced by 5-HT were −240 ± 21 beats min^{-1}, +30 ± 5 mmHg and −48 ± 2 mmHg, respectively (n = 6). 5-MeOT did not induce the Bezold-Jarisch reflex but did cause a transient hypertension and prolonged hypotension similar to that induced by 5-HT (+28 ± 4 and −49 ± 4 mmHg, respectively, $P > 0·05$, paired *t*-test in both cases, n = 6).

Effects of antagonists

SDZ 205-557, tropisetron, SB 204070 and granisetron had no effect on the 5-HT_2-receptor-mediated pressor response or the prolonged hypotension mediated by 5-HT_1-like

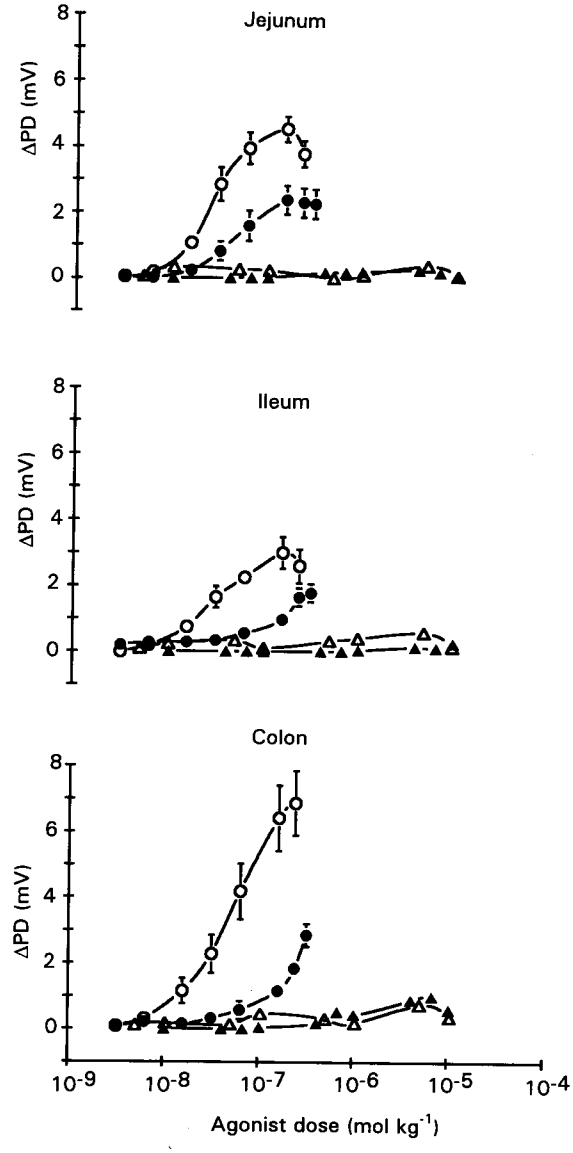

FIG. 1. Non-cumulative dose-response curves to 5-HT_4-receptor agonists in the jejunum, ileum and colon. Maximal change in transmural PD is plotted against agonist dose, each point being the mean ± s.e.m. of the number of observations in parentheses. ○ 5-HT (6), ● 5-MeOT (6), △ cisapride (6) and ▲ renzapride (4).

Table 1. Effect of 1 μmol kg^{-1} SDZ 205-557 (+ SDZ) on the secretory responses to 5-HT and 5-MeOT in the jejunum, ileum and colon.

	PD_{max} (mV)		ED50 value (nmol kg^{-1})	
	5-HT	+ SDZ 205-557	5-HT	+ SDZ 205-557
Jejunum	4·4 ± 0·5	4·0 ± 0·3	24 ± 4	34 ± 2
Ileum	4·2 ± 0·5	3·4 ± 0·5*	54 ± 10	73 ± 13*
Colon	6·4 ± 0·5	6·3 ± 0·2	49 ± 8	74 ± 18
	5-MeOT	+ SDZ 205-557	5-MeOT	+ SDZ 205-557
Jejunum	3·0 ± 0·5	3·5 ± 0·5	74 ± 9^{+++}	93 ± 17
Ileum	2·1 ± 0·5^{+}	2·4 ± 0·6	122 ± 11^{++}	110 ± 25
Colon	2·0 ± 0·2^{+++}	1·9 ± 0·3	110 ± 16^{++}	143 ± 22

Values correspond to the means of the PD_{max} and the ED50 values ± s.e.m. (n = 6). *$P < 0·05$, Student's paired t-test between the response with and without SDZ 205-557; $^{+}P < 0·05$,$^{++}P < 0·01$,$^{+++}P < 0·001$, Student's unpaired t-test between 5-HT and 5-MeOT.

receptors, nor did they alter the basal intestinal PD values ($P > 0·05$, n = 18 in each case). The secretory responses to acetylcholine in the jejunum, ileum and colon (control values: PD_{max} = 5·0 ± 0·3, 1·4 ± 0·2, 2·4 ± 0·2 mV; ED50 = 47 ± 5, 54 ± 5, 44 ± 5 nmol kg^{-1}, respectively, n = 18) were unaffected by any of the antagonists ($P > 0·05$ in all cases).

Tropisetron and SDZ 205-557 are dual 5-HT$_3$- and 5-HT$_4$-receptor antagonists, with tropisetron being approximately 100 times more potent at 5-HT$_3$ receptors, while SDZ 205-557 is approximately 10 times more potent at 5-HT$_4$ receptors (Buchheit et al 1992). To estimate the appropriate dose to antagonize 5-HT$_4$ receptors their effects on the 5-HT$_3$ receptor-mediated fall in heart rate were measured.

Tropisetron effectively antagonized the Bezold-Jarisch reflex at a dose of 10 nmol kg^{-1} (Fig. 3) and so a dose of 1 μmol kg^{-1} should also block 5-HT$_4$ receptors. This dose of tropisetron is supported by experiments in piglets (Saxena et al 1992). SDZ 205-557 significantly inhibited the fall in heart rate at a dose of 10 μmol kg^{-1}, with 1 μmol kg^{-1} being without effect (Fig. 3). Thus a dose of 1 μmol kg^{-1} should antagonize 5-HT$_4$ receptors without affecting 5-HT$_3$ receptors. SB 204070, at a dose of 15 nmol kg^{-1}, had no effect on the 5-HT$_3$-receptor-mediated fall in heart rate.

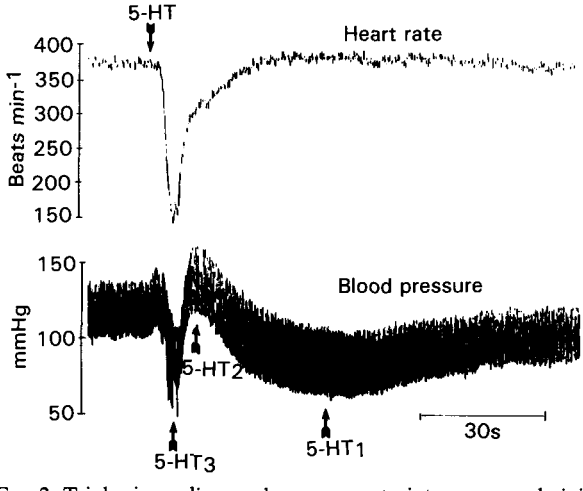

FIG. 2. Triphasic cardiovascular response to intravenous administration of 0·16 μmol kg^{-1} 5-HT. Upper trace shows heart rate (beats min^{-1}) and lower trace shows blood pressure (mmHg). Each phase is mediated by a different 5-HT receptor type, 5-HT$_3$, 5-HT$_2$ and 5-HT$_1$ receptor as indicated by the arrows.

Effect of SDZ 205-557

SDZ 205-557 (1 μmol kg^{-1}) reduced the secretory response

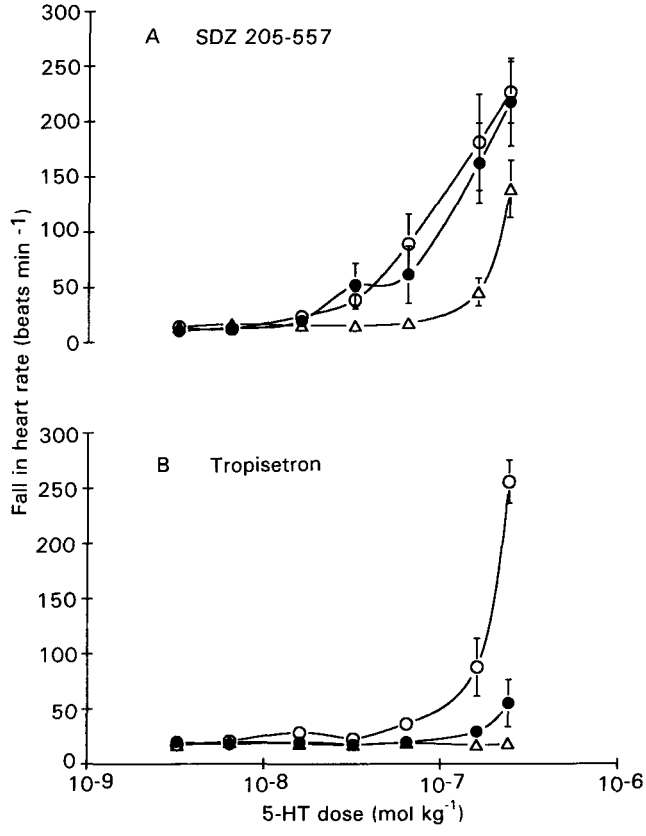

FIG. 3. Effect of SDZ 205-557 and tropisetron on the non-cumulative dose-response curve of the 5-HT$_3$-receptor mediated fall in heart rate. Each point is the mean ± s.e.m. of the number of observations in parenthesis. ○ Control (n = 6 in both cases); A: ● + 1 μmol kg^{-1} SDZ 205-557 (6), △ + 10 μmol kg^{-1} SDZ 205-557 (6); B: ● + 10 nmol kg^{-1} tropisetron (6), △ + 1 μmol kg^{-1} tropisetron (6).

Table 2. Effect of tropisetron on the 5-HT secretory response in the jejunum, ileum and colon.

	PD_{max} (mV)		
	Control	+ Tropisetron (10 nmol kg^{-1})	+Tropisetron (1 μmol kg^{-1})
Jejunum	4·5 ± 0·6	4·2 ± 0·5*	3·4 ± 0·5***
Ileum	4·1 ± 0·5	3·1 ± 0·6*	2·5 ± 0·5**
Colon	5·3 ± 0·7	5·6 ± 0·5	2·2 ± 0·3*
	ED50 (nmol kg^{-1})		
	Control	+ Tropisetron (10 nmol kg^{-1})	+Tropisetron (1 μmol kg^{-1})
Jejumun	28 ± 3	42 ± 5*	49 ± 5**
Ileum	31 ± 6	51 ± 6**	72 ± 21*
Colon	23 ± 3	118 ± 15***	101 ± 20*

Values correspond to the means of the PD_{max} and the ED50 values ± s.e.m. (n = 6). *$P < 0·05$, **$P < 0·01$, ***$P < 0·001$, Student's paired t-test.

to 5-HT in the ileum, but had no effect on the jejunal response. The ED50 value for 5-HT was increased in the colon, although this did not reach significance (Table 1).

The same dose of SDZ 205-557 had no effect on the 5-MeOT-induced rise in transmural PD in any intestinal region (Table 1).

SDZ 205-557 (10 μmol kg^{-1}) potentiated the secretory response to 5-MeOT in the jejunum (PD_{max} control: 2·8 ± 0·2 mV; +SDZ 205-557; 3·4 ± 0·1 mV, n = 6, $P < 0·05$, paired t-test) and tended to inhibit the ileal and colonic responses, increasing the ED50 from 86 ± 17 to 201 ± 41 nmol kg^{-1} and from 113 ± 31 to 207 ± 49 nmol kg^{-1}, respectively (n = 6). These changes however, failed to reach significance ($P > 0·05$ paired t-test in both cases).

Effect of tropisetron

Tropisetron (10 nmol kg^{-1}) significantly inhibited the secretory response to 5-HT in each of the three regions, with the higher dose of 1 μmol kg^{-1} causing a further reduction in the response (Table 2).

Tropisetron (1 μmol kg^{-1}) had no inhibitory effect on the PD_{max} induced by 5-MeOT in any region (ileal and colonic responses $P > 0·05$) and actually increased the PD_{max} in the jejunum (from 2·5 ± 0·4 to 2·8 ± 0·4 mV, $P < 0·01$). It did, however, increase the ED50 value in the jejunum and colon from 45 ± 4 to 81 ± 6 nmol kg^{-1} ($P < 0·05$) and 191 ± 42 to 371 ± 10 nmol kg^{-1} ($P < 0·01$), respectively, with no change in the ileum (control: 118 ± 33; + tropisetron: 155 ± 43 nmol kg^{-1}, $P > 0·05$, paired t-test, n = 6 in each case).

Effect of SB 204070

The selective 5-HT_4-receptor antagonist SB 204070 (Wardle et al 1993) at the effective dose of 15 nmol kg^{-1} (Bingham et al 1993) had no effect on the jejunal secretory response to 5-HT, but did decrease the PD_{max} in the ileum (from 3·7 ± 0·8 to 1·5 ± 0·4 mV $P < 0·05$) and colon (from 5·6 ± 0·5 to 5·0 ± 0·5 mV, $P < 0·05$). In the colon the ED50 value was also increased from 56 ± 10 to 78 ± 7 nmol kg^{-1} ($P < 0·05$, paired t-test, n = 6 in each case).

Effect of granisetron

The 5-HT_3-receptor antagonist granisetron (0·9 μmol kg^{-1}) decreased the secretory response to 5-HT in each of the three regions (Table 3). This dose of granisetron abolished the Bezold-Jarisch reflex decreasing the bradycardia from −254 ± 13 to −28 ± 4 beats min^{-1} ($P < 0·001$, paired t-test, n = 6). No further inhibition was obtained with 9 μmol kg^{-1}.

Granisetron had no inhibitory effect on the 5-MeOT-induced rise in transmural PD in any of the regions of gut. It did, however, potentiate the jejunal response to 5-MeOT by significantly increasing the PD_{max} (Table 4).

Discussion

Although the ability of 5-HT to induce secretion throughout the intestinal tract is well established, no one receptor subtype has been identified as being responsible. The recent characterization of an additional receptor subtype, the 5-HT_4 receptor (Humphrey et al 1993), has raised the possibility that this might be involved in the 5-HT-induced secretion in the intestine. A number of agents have now been identified as agonists at the 5-HT_4 receptor. These include 5-MeOT (Bockaert et al 1992) and the substituted benzamides such as cisapride and renzapride (Dumuis et al 1989). When their ability to stimulate intestinal secretion was tested, it was clear that there was no consistent effect. 5-MeOT induced a secretory response in the jejunum, ileum and colon, although in all cases it was less effective than 5-HT. In addition to its effects on the 5-HT_4 receptor, 5-MeOT is also able to activate 5-HT_1-like and 5-HT_{2A}-like receptors. This was confirmed in the present study by its actions on blood pressure, where it caused a 5-HT_{2A}-like receptor-mediated pressor phase followed by a 5-HT_1-like receptor-mediated prolonged depressor response. Thus its ability to mimic the secretory actions of 5-HT does not necessarily indicate that 5-HT_4 receptors are involved in

Table 3. Effect of granisetron (0·9 μmol kg^{-1}) on the 5-HT secretory response in the jejunum, ileum and colon.

	PD_{max} (mV)		ED50 (nmol kg^{-1})	
	Control	+ Granisetron	Control	+ Granisetron
Jejunum	5·3 ± 0·3	2·2 ± 0·3***	30 ± 6	53 ± 6***
Ileum	3·3 ± 0·4	2·7 ± 0·3*	42 ± 3	71 ± 5***
Colon	5·9 ± 0·5	4·7 ± 0·6*	47 ± 3	89 ± 8***

Values correspond to the means of the PD_{max} and the ED50 values ± s.e.m. (n = 12), *$P < 0·05$, ***$P < 0·001$, Student's paired t-test.

Table 4. Effect of 0·9 μmol kg^{-1} granisetron on the secretory response to 5-MeOT in the jejunum, ileum and colon.

	PD_{max} (mV)		ED50 (nmol kg^{-1})	
	5-MeOT	+ Granisetron	5-MeOT	+ Granisetron
Jejunum	2·9 ± 0·3	3·6 ± 0·3***	64 ± 14	75 ± 10
Ileum	1·8 ± 0·4	1·7 ± 0·5	111 ± 29	126 ± 29
Colon	3·5 ± 0·5	3·4 ± 0·5	188 ± 36	193 ± 42

Values correspond to the means of the PD_{max} and ED50 values ± s.e.m.(n = 6), ***$P < 0·001$, Student's paired t-test.

the regulation of intestinal transport. The substituted benzamides cisapride and renzapride failed to increase transintestinal PD in any intestinal region investigated, suggesting that 5-HT$_4$ receptors do not contribute to the electrogenic Cl^- secretory response of the gut. Although these agents are usually considered to be only partial agonists at the 5-HT$_4$ receptor (Baxter et al 1991), it might be anticipated that at least some response should have been observed.

Further investigation of 5-HT$_4$-receptor involvement in the intestinal secretory response to 5-HT was carried out using antagonists. Most agents that block 5-HT$_4$ receptors also have antagonistic actions at 5-HT$_3$ receptors, although it is possible to separate effects at the two receptors by differential dose levels. Appropriate doses of tropisetron and SDZ 205-557 were determined from measurements of their effects on the 5-HT$_3$-receptor-mediated bradycardia. At high doses tropisetron antagonizes 5-HT$_4$ receptors in addition to its inhibition of 5-HT$_3$ receptors (Buchheit et al 1992). This agent caused a dose-dependent reduction in the maximal PD change induced by 5-HT and also increased the ED50 values (Table 2). Much of its effects, however, could be attributed to an inhibition of 5-HT$_3$ receptors since granisetron caused similar changes (Table 3). Also tropisetron had no inhibitory effect on the PD_{max} induced by 5-MeOT (an agonist that lacks affinity for 5-HT$_3$ receptors) in any region, and even caused a potentiation in the jejunum. However, when the ED50 values are examined, tropisetron was found to inhibit the colonic response to 5-MeOT, suggesting that 5-HT$_4$ receptors may make some contribution to the control of secretion in the colon.

The potentiation of the jejunal PD_{max} to 5-MeOT was also seen with granisetron (Table 3) and 10 μmol kg^{-1} SDZ 205-557. It is difficult to explain why a 5-HT$_3$-receptor antagonist should influence the response to an agonist with no 5-HT$_3$-receptor activity, but it is possible that 5-MeOT may induce local 5-HT release which then acts on 5-HT$_3$ receptors attenuating the secretory response. Abolition of such an inhibitory component by granisetron would explain the enhanced response to 5-MeOT in the presence of this 5-HT$_3$-receptor antagonist. Since cholinergic neurons contribute to the secretory response to 5-HT (Franks et al 1993) and 5-MeOT (unpublished data) in the rat jejunum, the neurally-released acetylcholine may stimulate 5-HT release from enterochromaffin cells by activation of muscarinic M_3 receptors (Reimann et al 1993). Enhancement of the response to 5-MeOT by SDZ 205-557 and tropisetron has also been demonstrated in the tunica muscularis mucosae of rat oesophagus (Waikar et al 1993). This was postulated to be due to endogenous 5-HT release as the antagonists showed no allosteric interaction.

Tropisetron was able to reduce the secretory response to 5-HT further than granisetron in the ileum and colon. This may be due to its ability to inhibit two different 5-HT-receptor subtypes. It has been shown that inhibition of 5-HT$_2$ or 5-HT$_3$ receptors partially reduces cholera toxin-induced fluid secretion, but by inhibiting both receptors the secretion is totally abolished (Beubler & Horina 1990). This would suggest that the secretory response to 5-HT is mediated by more than one receptor, and that these receptors can act independently of each other.

SDZ 205-557 is more effective at 5-HT$_4$ receptors than at 5-HT$_3$ receptors in the guinea-pig (Buchheit et al 1992). At doses that failed to inhibit the 5-HT$_3$-receptor-mediated bradycardia, SDZ 205-557 did not influence the secretory response to 5-HT in either the jejunum or the colon, although it did cause a small inhibition in the ileum. It did not, however, cause a reduction in the response to 5-MeOT in any intestinal region tested (Table 1).

Recent investigations have shown that whilst in the guinea-pig, SDZ 205-557 is more selective for 5-HT$_4$ receptors, this is not the case in the rat where the selectivity for 5-HT$_3$ and 5-HT$_4$ receptors is similar (Eglen et al 1993). Therefore, 1 μmol kg^{-1} SDZ 205-557 may not have been sufficient to fully antagonize 5-HT$_4$ receptors. Using the higher dose of 10 μmol kg^{-1} SDZ 205-557 (which inhibited the 5-HT$_3$ receptor-mediated bradycardia), an insignificant rightward shift in the ileal and colonic response to 5-MeOT was observed. The jejunal PD_{max} response to 5-MeOT was augmented as with tropisetron and granisetron. Thus even using the higher dose there is only a hint of 5-HT$_4$ receptor involvement in the ileum and colon.

Although SDZ 205-557 was reported to have a half-life of only 23 min in-vivo (Eglen et al 1993), in our preparation 10 μmol kg^{-1} SDZ 205-557 inhibited the Bezold-Jarisch reflex induced by the last dose of 5-HT in the dose-response curve 15 min after application, indicating its continued effectiveness.

SB 204070 is a more selective 5-HT$_4$-receptor antagonist than either tropisetron or SDZ 205-557, having a 5000-fold selectivity for the 5-HT$_4$ receptor (Wardle et al 1993). This was confirmed by its failure to influence any of the phases of the 5-HT-induced change in cardiovascular state. It did not alter the secretory response of the jejunum to 5-HT, but caused a small inhibition of the ileal and colonic responses.

The inhibitions observed with the antagonists could not be attributed to a direct action on the secretory process itself, since they had no effect on the response to acetylcholine. Acetylcholine stimulates the same secretory process as 5-HT, and in both cases the intracellular signalling pathway that leads to its activation is considered to involve an elevation of intracellular Ca^{2+} (Donowitz et al 1980, 1982).

The present investigation suggests that it is unlikely that 5-HT$_4$ receptors make any contribution to the secretory response of the jejunum to 5-HT, although they may play a small role in both the ileal and colonic responses. The discrepancy between our findings and those of others in the human and guinea-pig intestine (Budhoo & Kellum 1993; Burleigh & Borman 1993; Leung et al 1993) probably

represents inter-species differences. In addition, the present study was carried out in-vivo, measuring transintestinal PDs whereas in the other studies SCC was measured in-vitro.

This study demonstrates, that, in-vivo, 5-HT_4 receptors play only a minor role in the secretory response of the ileum and colon to stimulation by 5-HT, while in the jejunum there is no evidence for their participation. 5-HT_3 receptors contribute to the response in all regions of the intestine, but are not solely responsible. It is likely that the intestinal secretory response to 5-HT is a complex one which may involve more than one 5-HT receptor subtype.

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