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Several Receptor Subtypes Contribute to 5-Hydroxytryptamine-induced Secretion by Rat Ileum In-vitro

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Abstract

The receptors contributing to 5-hydroxytryptamine (5-HT)-induced secretion by rat ileum were investigated in-vitro using selective agonists and antagonists.

5-HT induced a dose-dependent increase in the short-circuit current (SCC) generated by both intact and stripped sheets of rat ileum. 1-Phenylbiguanide, a selective 5-HT_3 agonist, and 5-methoxytryptamine, an agonist that lacks affinity for 5-HT_3 receptors, also increased the SCC. In intact sheets 5-HT was more effective than either 1-phenylbiguanide or 5-methoxytryptamine, whereas in stripped sheets 5-HT and 5-methoxytryptamine were equipotent, with 1-phenylbiguanide having little effect. Tetrodotoxin abolished the response of intact sheets to 1-phenylbiguanide but only reduced the responses to 5-HT and 5-methoxytryptamine by 57% and 54%, respectively. This inhibition was reduced to 25% in stripped sheets. The 5-HT_3 antagonist granisetron abolished the response to 1-phenylbiguanide, but did not alter the effects of 5-HT. Ketanserin, a 5-HT_2 antagonist, had a small effect on the actions of 5-methoxytryptamine in intact, but not stripped, sheets and no effect on the response to 5-HT in either preparation. Tropisetron, a 5-HT_3 and 5-HT_4 antagonist, inhibited the response to 5-methoxytryptamine, but had less effect on the response to 5-HT. Desensitization to 1-phenylbiguanide inhibited the response to 5-HT in intact, but not stripped sheets, whereas desensitization to 5-methoxytryptamine abolished the 5-HT response in stripped sheets, but induced only 42% inhibition in intact sheets. Previous exposure to a combination of both 1-phenylbiguanide and 5-methoxytryptamine abolished the 5-HT-induced increase in SCC in both preparations.

The findings suggest that 5-HT-induced ileal secretion involves more than one 5-HT receptor subtype and that both neural and non-neural mechanisms contribute to the response.

5-Hydroxytryptamine (5-HT) is abundant in the intestinal tract where it is found not only in the enterochromaffin cells of the mucosa, but also in both neural and immune elements of the subepithelial tissues (McKay & Perdue 1993). It is well-established that 5-HT induces a net secretion of fluid and electrolytes by the small intestine (Kisloff & Moore 1976; Donowitz et al 1977) which is a consequence of inhibition of electroneutral NaCl absorption together with stimulation of electrogenic Cl^- secretion (Hardcastle et al 1981). However, the mechanisms responsible for inducing these changes in the ion transport activity of the enterocytes that line the intestinal lumen have yet to be fully elucidated. The complexity of the 5-HT receptor population, which comprises numerous subtypes (Bradley et al 1986; Hoyer & Schoeffter 1991; Hoyer et al 1994), and the multiplicity of possible sites of 5-HT action (Cooke 1994) contribute to this uncertainty. Moreover, the situation is further complicated not only by species differences (McLean et al 1993) but also by regional variations in the way the intestine responds to 5-HT. Such differences have recently been demonstrated in the small intestine of both the rat (Franks et al 1995, 1996; Hardcastle & Hardcastle 1996b) and the pig (Grondahl et al 1996) and also in the rat colon (Ayton et al 1995). These problems have resulted in the lack of a consensus concerning the mechanism of 5-HT action on intestinal transport processes.

Recent in-vivo studies have furnished evidence that several different 5-HT receptor subtypes contribute to 5-HT-induced secretion in rat small intestine (Hardcastle & Hardcastle 1995) and the current study was designed to investigate whether these observations could be extended to the in-vitro situation. Preliminary reports of these studies have been presented (Hardcastle & Hardcastle 1996a, 1997).

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Materials and Methods

Chemicals

5-Hydroxytryptamine creatinine sulphate, 5-methoxytryptamine and tetrodotoxin were obtained from Sigma (Poole, Dorset, UK), and 1-phenylbiguanide from Aldrich (Gillingham, Dorset, UK). Granisetron (BRL43694) and tropisetron (ICS 205 930) were gifts from SmithKline Beecham Pharmaceuticals (Harlow, UK) and ketanserin was donated by Janssen Pharmaceutica (Beerse, Belgium). All drugs were dissolved in 154 mM NaCl except for tropisetron, the stock solution of which (1·4 mg mL^{-1}) was prepared with 0·1 mL 1 M HCl + 0·9 mL 154 mM NaCl and diluted subsequently with 154 mM NaCl.

Animals

Experiments were performed on male Wistar rats, 230–250 g, obtained from the Sheffield Field Laboratories and allowed free access to food and water. They were anaesthetized with sodium pentobarbitone (Sagatal, 60 mg kg^{-1} i.p.).

Measurement of transintestinal electrical activity across ileal sheets

The potential difference (PD), short-circuit current (SCC) and tissue resistance were measured across paired sheets of intact and stripped (outer muscle layers and myenteric plexus

removed) ileum taken from the region immediately proximal to the terminal 5 cm of the small intestine. Each sheet was mounted in an Ussing chamber with an aperture of 1·925 cm^2 and incubated at 37°C in Krebs bicarbonate saline oxygenated with 95% O_2–5% CO_2. The serosal fluid contained 10 mM glucose and the mucosal fluid 10 mM mannitol; the volume of each was 5 mL. The PD was measured using salt-bridge electrodes connected via calomel half-cells to a differential input electrometer with output to a two-channel chart recorder (Linseis L6512). Current was applied across the tissue via conductive plastic electrodes and tissue resistance determined from the PD change induced by a 100 μA current pulse, taking into account the fluid resistance. The initial resistances of each tissue pair did not differ by more than 25%. The SCC generated by the sheets was calculated from PD and resistance measurements by use of Ohm's law.

Tissues were left to stabilize for 10 min after mounting and then readings of electrical activity were taken at 1-min intervals. After 5 min basal readings 5-HT agonists were added to the serosal solution. Cumulative concentration–response curves were constructed by applying the next concentration of agonist at the peak of the response to the previous application as described by Bunce et al (1991). When the effects of an antagonist or tetrodotoxin were investigated the drug was added to the serosal solution of the test sheet at the concentration indicated as soon as the sheets were set up, with control sheets receiving an equivalent volume (2% v/v) of vehicle. Ten minutes after the final addition of agonist, glucose (10 mM) was added to the mucosal solution of both sheets to test tissue viability and possible non-specific actions of the test conditions. The effects of desensitization were investigated by making two consecutive additions of agonist at 10-min intervals, and after a further 10 min determining the response to 5-HT. Glucose (10 mM) was again added at the end of the experiment.

Expression of results

Results are expressed as mean values ± s.e.m. A *t*-test, paired or unpaired as appropriate, was used to assess the significance of any differences observed. EC50 values (concentrations inducing half the maximum response) were calculated as geometric means (95% confidence limits) and statistical analysis was performed on log-transformed data.

Results

Ileal response to 5-HT agonists

Intact sheets of rat ileum generated a basal PD of 1·1 ± 0·1 mV, an SCC of 16·9 ± 1·3 μA cm^{-2}, serosa positive, and a tissue resistance of 62·8 ± 1·5 ohm cm^2 (n = 170). Stripped sheets (n = 143) had higher basal PD (1·3 ± 0·1 mV, $P < 0·05$) and SCC (24·8 ±1·4 μA cm^{-2}, $P < 0·001$) values, with a lower tissue resistance (55·5 ± 1·2 ohm cm^2, $P < 0·001$). 5-HT (100 μM) increased the SCC in both intact and stripped preparations and this effect was mimicked by both 1-phenylbiguanide, a selective 5-HT_3 agonist (Hoyer et al 1994), and 5-methoxytryptamine, an agonist that lacks affinity for 5-HT_3 receptors (Fozard 1985; Leff & Martin 1988; Craig et al 1990; Fig. 1). In the intact preparation 5-HT induced a much larger response than equimolar concentrations of 5-methoxytryptamine and 1-phenylbiguanide but in the stripped preparation 5-methoxytryptamine was as effective as 5-HT, with 1-phenylbiguanide having little effect. The effects of these agonists were concentration-dependent (Fig. 2). 5-HT induced similar maximum responses in both preparations ($P > 0·05$), but stripped sheets were more sensitive, with a significantly lower EC50 value (Table 1). 5-Methoxytryptamine exhibited a similar increase in sensitivity in the stripped preparation and, in addition, the maximum response was greater (Table 1). As 1-phenylbiguanide elicited such a small increase in SCC in stripped sheets concentration–response curves were not constructed for this agonist.

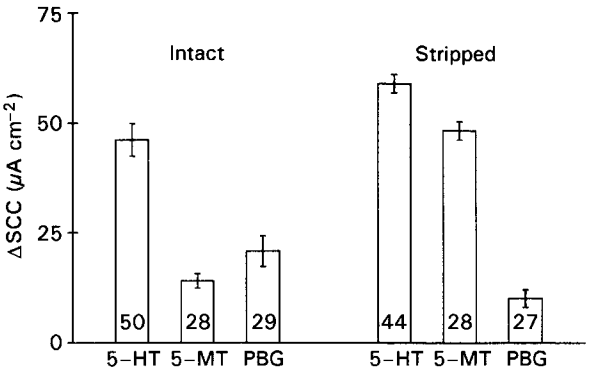

FIG. 1. Effects of 5-HT, 5-methoxytryptamine (5-MT) and 1-phenylbiguanide (PBG) on the SCC generated by intact and stripped sheets of rat ileum. Agonists were added to the serosal solution to give a final concentration of 100 μM and the maximum increase in SCC (ΔSCC) was recorded. Each bar represents the mean ± s.e.m. of the number of observations indicated.

Effects of tetrodotoxin on the responses to 5-HT agonists

In intact sheets tetrodotoxin induced a 37% reduction in basal SCC (control 17·8 ± 4·9 μA cm^{-2}; +tetrodotoxin 11·2 ± 3·1 μA cm^{-2}, $P < 0·05$, n = 12) and inhibited the response to 5-HT by 57% and to 5-methoxytryptamine by 54% (Fig. 3). In contrast, the effects of 1-phenylbiguanide were abolished in the presence of the neurotoxin (Fig. 3). The increase in SCC induced by glucose was unaffected by tetrodotoxin (control 121·0 ± 14·4 μA cm^{-2}; +tetrodotoxin 107·9 ± 11·8 μA cm^{-2}, $P > 0·05$, n = 12). In stripped sheets the basal SCC was unaffected by tetrodotoxin (control 19·5 ± 2·4 μA cm^{-2}; +tetrodotoxin 18·7 ± 2·5 μA cm^{-2}, $P > 0·05$, n = 8) and the inhibition of the 5-HT response was reduced to 25% ($P < 0·05$). The glucose-dependent increase in SCC was again unaffected by tetrodotoxin (control 130·1 ± 14·6 μA cm^{-2}; + tetrodotoxin 134·2 ± 18·8 μA cm^{-2}, $P > 0·05$, n = 8). The residual response to 5-HT in the presence of tetrodotoxin was greater in the stripped preparation (intact 25·9 ± 2·3 μA cm^{-2}, n = 4; stripped 44·2 ± 1·3 μA cm^{-2}, n = 8, $P < 0·001$), although in the absence of the neurotoxin there was no difference between the magnitudes of the responses to 5-HT in the two preparations ($P > 0·05$).

Effects of 5-HT antagonists

The 5-HT_3 antagonist granisetron (Sanger & Nelson 1989) completely abolished the effects of 1-phenylbiguanide in intact sheets, but at the same concentration (1·4 μM) it had no effect on the response to 5-HT in either intact or stripped preparations (Fig. 4, Table 2).

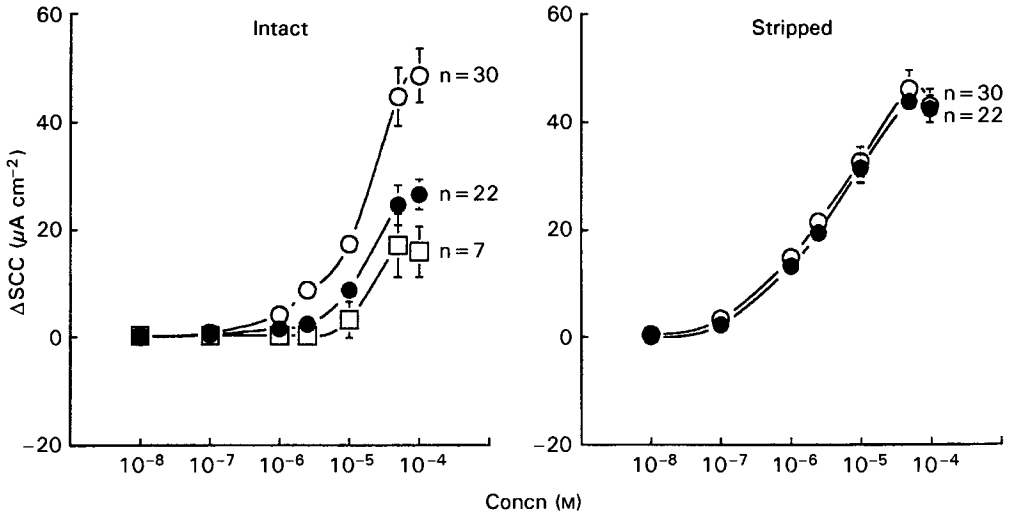

FIG. 2. Concentration-dependence of 5-HT agonist action in intact and stripped sheets of rat ileum. The increases in SCC (ΔSCC) induced by cumulative additions of 5-HT (○), 5-methoxytryptamine (●) and 1-phenylbiguanide (□) are plotted as a function of log agonist concentration and each point represents the mean ± s.e.m. of the number of observations indicated. The error bar is sometimes smaller than the size of the symbol.

Table 1. Effects of 5-HT agonists on the SCC generated by intact and stripped sheets of rat ileum.

	n	Maximum increase in scc (μA cm^{-2})	EC50 (μM)
Intact sheets			
5-HT	30	49·8 ± 5·4	12·5 (9·7–16·1)
5-MT	22	28·6 ± 3·4*	20·0 (16·7–23·9)*
1-PB	7	18·0 ± 5·8*	1239 (128–12039)*
Stripped sheets			
5-HT	30	46·5 ± 3·5	3·2 (2·6–3·9)†
5-MT	22	43·9 ± 2·4†	3·3 (2·5–4·4)†

Cumulative concentration–response curves to 5-HT, 5-methoxytryptamine (5-MT) and 1-phenylbiguanide (PBG) were constructed. The increase in scc is expressed as the maximum response and each value represents the mean ± s.e.m. of the number of observations indicated. EC50 values (concentrations inducing half the maximum response) are geometric means (95% confidence limits). An unpaired t-test was used to assess the significance of any differences observed. *$P < 0.01$, comparison of 5-MT and PBG with 5-HT; †$P < 0.001$, comparison of stripped and intact preparations.

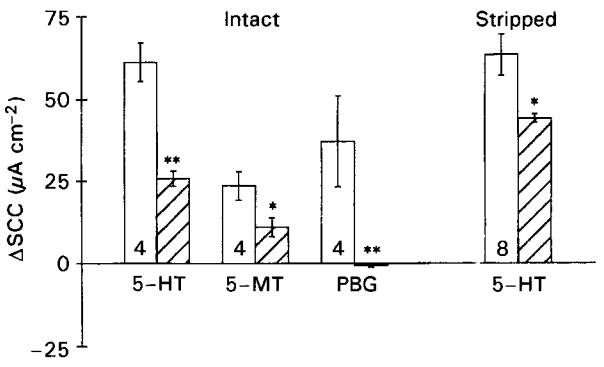

FIG. 3. Effect of serosal tetrodotoxin (TTX; 10 μM) on the increase in SCC (ΔSCC) induced by 100 μM 5-HT, 5-methoxytryptamine (5-MT) and 1-phenylbiguanide (PBG) in intact and stripped sheets of rat ileum. Each bar represents the mean ± s.e.m. of the number of observations indicated; a paired t-test was used to assess the significance of the action of TTX. □ Control, ▨ plus TTX. *$P < 0.05$; **$P < 0.001$.

Ketanserin, a 5-HT$_2$ antagonist (Bradley et al 1986), induced a small but significant increase in the EC50 value for 5-methoxytryptamine in intact sheets, but was without effect in stripped sheets (Table 2). It had no effect on the response to 5-HT in either preparation (Table 2).

Tropisetron, a 5-HT$_3$ and 5-HT$_4$ antagonist (Buchheit et al 1992), inhibited the response to 5-methoxytryptamine in both intact and stripped sheets (Fig. 5), increasing the EC50 in both preparations and reducing the maximum response in intact sheets. It also increased the EC50 for 5-HT in stripped sheets, but in intact sheets the EC50 was reduced (Table 2).

None of the antagonists tested affected the increase in SCC associated with active sodium-dependent glucose transport in either intact or stripped preparations ($P > 0.05$ in all cases).

Effects of desensitization

Repeated application of agonist to 5-HT receptors in-vitro leads to loss of response, the phenomenon denoted desensitization (Hubel 1984; Cooke & Carey 1985; Castro et al 1987; Hardcastle et al 1994). A second application of 1-phenylbiguanide failed to elicit an increase in SCC in either intact or stripped sheets (Table 3). A similar pattern was observed with 5-methoxytryptamine in stripped sheets, but in the intact preparation a second application of 5-methoxytryptamine still induced a significant ($P < 0.001$) increase in SCC although its magnitude was only 45% of the first response (Table 3). The response to 5-HT in intact sheets was reduced by desensitization to both 1-phenylbiguanide and 5-methoxytryptamine, but in stripped sheets 1-phenylbiguanide was without effect whereas 5-methoxytryptamine abolished the 5-HT response (Fig. 6). Previous exposure to a combination of both 1-phenylbiguanide and 5-methoxytryptamine eliminated the 5-HT-induced increase in SCC in both preparations (Fig. 6). Desensitization to 5-methoxytryptamine did not affect the response to 1-phenylbiguanide, nor did desensitization to 1-phenylbiguanide affect the response to 5-methoxytryptamine ($P > 0.05$ in both cases).

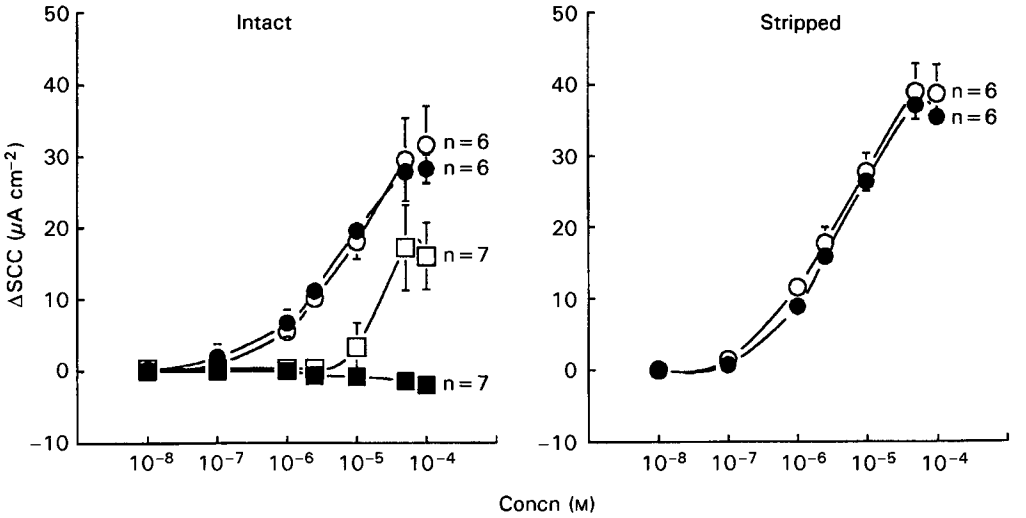

FIG. 4. Effects of granisetron (●, ■, 1·4 μM in the serosal solution) on the responses of intact and stripped sheets of rat ileum to 5-HT (○, ●) and 1-phenylbiguanide (□, ■). The increases in SCC (ΔSCC) induced by cumulative additions of 5-HT and PBG are plotted as a function of log agonist concentration and each point represents the mean ± s.e.m. of the number of observations indicated. The error bar is sometimes smaller than the size of the symbol.

Table 2. Effects of 5-HT antagonists on the responses of intact and stripped sheets of rat ileum to 5-HT, 5-methoxytryptamine (5-MT) and 1-phenylbiguanide (PBG).

	Intact			Stripped		
	n	Maximum increase in scc ($\mu A\ cm^{-2}$)	EC50 (μM)	n	Maximum increase in scc ($\mu A\ cm^{-2}$)	EC50 (μM)
5-HT	6	31·9 ± 5·7	6·0 (3·3–10·9)	6	39·9 ± 3·9	3·5 (2·9–4·2)
5-HT + granisetron		28·7 ± 1·8	3·2 (0·8–11·4)		37·2 ± 1·8	3·6 (2·6–4·9)
PBG	7	18·0 ± 5·8	1239 (128–12039)		–	–
PBG + granisetron		− 0·1 ± 0·1*	–		–	–
5-HT	8	73·3 ± 13·1	15·5 (10·3–23·7)	8	37·1 ± 2·9	6·0 (4·9–7·3)
5-HT + ketanserin		63·9 ± 12·6	13·7 (7·4–25·3)		35·1 ± 3·3	5·4 (4·7–6·2)
5-MT	6	24·2 ± 2·2	18·8 (14·4–24·4)	6	42·0 ± 3·6	3·6 (2·3–5·8)
5-MT + ketanserin		22·8 ± 2·1	22·3 (17·3–28·7)*		39·8 ± 4·9	3·5 (1·6–7·6)
5-HT	8	39·0 ± 7·5	10·9 (6·2–19·1)	8	40·7 ± 5·5	2·0 (1·2–3·5)
5-HT + tropisetron		28·2 ± 1·9	5·7 (4·5–7·2)*		41·6 ± 3·4	2·9 (1·8–4·5)*
5-MT	8	22·3 ± 1·9	18·2 (13·0–25·5)	8	43·1 ± 4·6	2·4 (1·2–4·8)
5-MT + tropisetron		15·1 ± 1·3*	37·6 (18·6–76·0)*		37·1 ± 1·6	12·7 (11·0–14·6)†

Granisetron (1·4 μM), ketanserin (2·5 μM) or tropisetron (5 μM) were present in the serosal solution of test sheets, while control sheets received an equivalent volume of vehicle. The increase in SCC is expressed as the maximum response and each value represents the mean ± s.e.m. of the number of observations indicated. EC50 values (concentrations inducing half the maximum response) are geometric means (95% confidence limits). A paired *t*-test was used to assess the significance of antagonist action. $*P < 0.05$, $\dagger P < 0.001$.

Discussion

The SCC generated both by intact and stripped sheets of rat ileum was increased not only by 5-HT but also by 1-phenylbiguanide, the selective 5-HT_3 agonist, and 5-methoxytryptamine, an agonist at 5-HT_1, 5-HT_2 and 5-HT_4 receptors, but not at 5-HT_3 receptors. This suggests that in addition to 5-HT_3 receptors, other 5-HT receptor subtypes must contribute to the secretory response. The role played by 5-HT_3 receptors was further investigated by examining the effect of the selective 5-HT_3 antagonist granisetron. At a concentration that totally abolished the response to 1-phenylbiguanide, granisetron had no effect on the increase in SCC induced by 5-HT itself in either intact or stripped preparations. A similar discrepancy has been observed in-vivo where granisetron increased the ED50 value for the selective 5-HT_3 agonist 2-methyl-5-HT by a factor of 11·8, while causing only a 1.8-fold increase in the value for 5-HT (Hardcastle & Hardcastle 1995). This supports the view that 5-HT_3 receptors cannot be solely responsible for 5-HT-induced ileal secretion.

5-Methoxytryptamine was much more effective in activating a secretory response in stripped preparations of rat ileum. At least part of its effects were mediated by 5-HT_4 receptors, because tropisetron, a 5-HT_3 and 5-HT_4 antagonist, increased the EC50 value by a factor of 2·1 in intact sheets and 5·3 in stripped sheets. It did not, however, increase the EC50 value for 5-HT in intact ileal sheets, although it did cause a small, but significant, 1·5-fold increase in stripped sheets. The observation that tropisetron is more effective in antagonizing the actions of 5-methoxytryptamine has also been reported for stripped sheets of guinea-pig ileum (Scott et al 1992) and pig jejunum (Hansen 1994), although only one concentration of

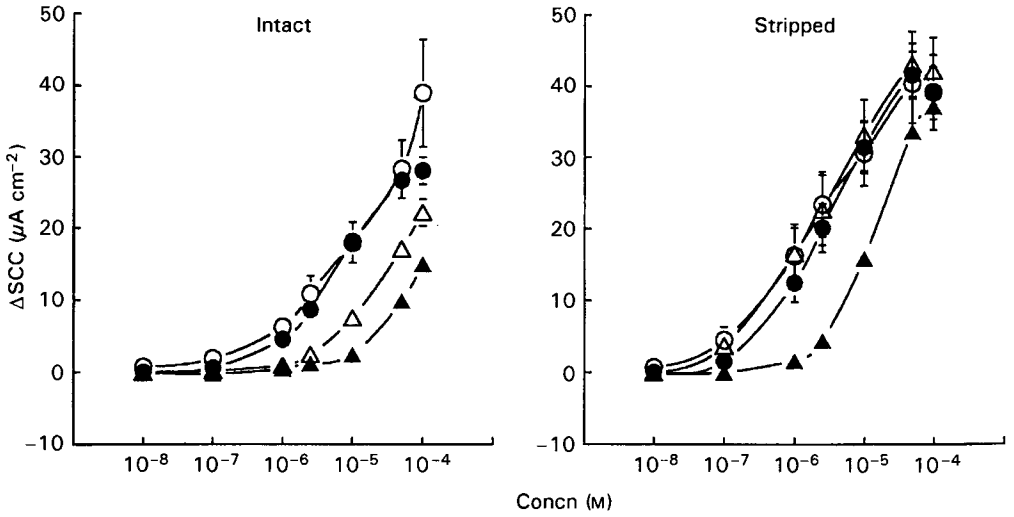

FIG. 5. Effects of tropisetron (●, ▲, 5·0 μM in the serosal solution) on the responses of intact and stripped sheets of rat ileum to 5-HT (○, ●) and 5-methoxytryptamine (△, ▲). The increases in SCC (ΔSCC) induced by cumulative additions of 5-HT and 5-MT are plotted as a function of log agonist concentration and each point represents the mean ± s.e.m. of 8 observations. The error bar is sometimes smaller than the size of the symbol.

Table 3. Desensitization of the response to 5-HT agonists.

		Increase in scc (μA cm^{-2})	
	n	1st application	2nd application
Intact sheets			
1-Phenylbiguanide	12	39·0 ± 7·7	− 1·4 ± 1·0†
5-Methoxytryptamine	12	10·3 ± 1·4	4·6 ± 0·5*
1-Phenylbiguanide + 5-Methoxytryptamine	8	43·0 ± 4·8	− 0·1 ± 1·0†
Stripped sheets			
1-Phenylbiguanide	12	6·1 ± 2·0	− 0·1 ± 0·2*
5-Methoxytryptamine	12	53·6 ± 3·9	− 3·7 ± 0·6†
1-Phenylbiguanide + 5-Methoxytryptamine	8	54·5 ± 4·2	− 4·8 ± 0·9†

Test sheets received two consecutive applications of agonist (both at a concentration of 100 μM) at 10-min intervals whereas control sheets received equivalent volumes of vehicle (2% v/v). The increases in scc induced by 5-HT agonists are given as the mean ± s.e.m. of the number of observations indicated; a paired *t*-test was used to compare the first and second responses. $*P < 0.01$, $\dagger P < 0.001$. Addition of vehicle had no effect on the scc ($P > 0.05$ in all cases).

agonist was tested by Hansen. Similarly a small rightward shift in the 5-HT dose–response curve induced by tropisetron has been observed in stripped preparations of the jejunum (Budhoo & Kellum 1994) and ileum (Borman & Burleigh 1993; Burleigh & Borman 1993) from man and rat distal colon (Bunce et al 1991; Siriwardena et al 1991; Budhoo et al 1996). Moreover, tropisetron inhibits 5-HT-induced fluid secretion in rat jejunum in-vivo (Beubler & Horina 1990; Beubler et al 1993). That tropisetron, an antagonist at both 5-HT_3 and 5-HT_4 receptors, inhibited the response to 5-methoxytryptamine, an agonist that lacks affinity for 5-HT_3 receptors, implicates 5-HT_4 receptors in the ileal secretory response to 5-HT stimulation.

The 5-HT_2 antagonist ketanserin had no effect on the response to 5-HT in either intact or stripped preparations of rat ileum; this is consistent with previous observations in both rat (Beubler et al 1990; Hardcastle & Hardcastle 1995) and guinea-pig (Scott et al 1992) small intestine and rat colon (Bunce et al 1991). Other reports however, suggest that ketanserin can inhibit 5-HT-induced secretion in rat colon (Siriwardena & Kellum 1993). It has also been shown to reduce 5-HT-stimulated fluid secretion in rat jejunum (Beubler & Horina 1990; Beubler et al 1990, 1993). In the current study ketanserin induced a small inhibition of the SCC response to 5-methoxytryptamine in intact sheets, but had no effect in stripped sheets. It also inhibits the 5-methoxytryptamine-induced increase in transintestinal electrical activity in-vivo (Hardcastle & Hardcastle 1995). The involvement of 5-HT_2 receptors in 5-HT-induced intestinal secretion remains unclear, but it is possible that they might play a small role in the overall secretory response to 5-HT challenge.

High concentrations of 1-phenylbiguanide and 5-methoxytryptamine do not seem to lose their selectivity, because desensitization to one of these agonists had no effect on the response to the other. Desensitization experiments also provided further information about the 5-HT receptors mediating the ileal secretory response, an approach that has previously been used in the investigation of 5-HT-induced changes in intestinal motility (Craig et al 1990). In the current study desensitization to 1-phenylbiguanide reduced the response to 5-HT in intact sheets, but had no effect in stripped sheets,

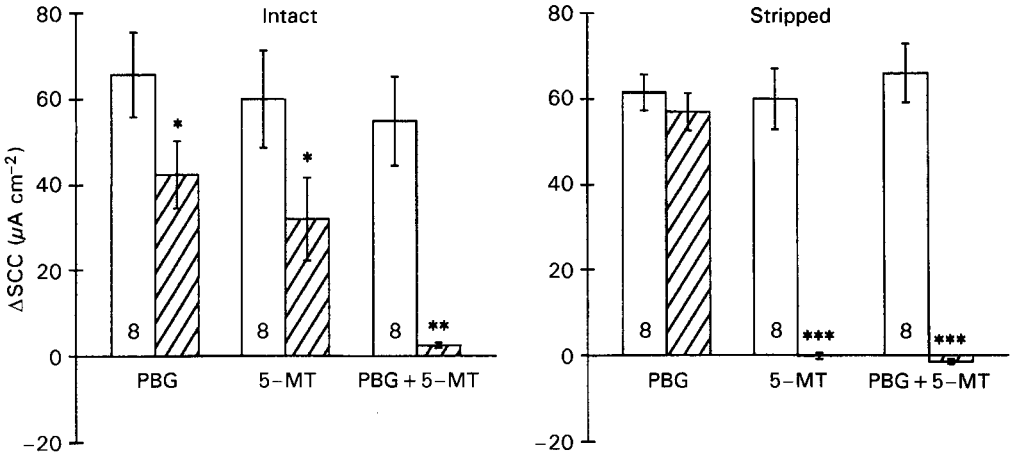

FIG. 6. Response of intact and stripped sheets of rat ileum to 100 μM 5-HT after previous exposure to two consecutive applications of 1-phenylbiguanide (PBG), 5-methoxytryptamine (5-MT) or PBG + 5-MT (100 μM in each case). Control sheets received an equivalent volume of vehicle (2% v/v). □ Control; ▨ test. Each bar represents the mean ± s.e.m. of the number of observations indicated and a paired t-test was used to compare the responses in control and test sheets. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

consistent with the relative lack of effect of 1-phenylbiguanide in the stripped preparation. In contrast, 5-methoxytryptamine desensitization was much more effective in inhibiting the action of 5-HT in stripped sheets. This reinforces the view that 5-HT-induced ileal secretion cannot be attributed to a single 5-HT receptor subtype.

The involvement of neural mechanisms in 5-HT-induced ileal secretion was investigated by examining the effects of tetrodotoxin on the increases in SCC that occurred in response to the application of 5-HT agonists. The effect of 1-phenylbiguanide was totally abolished by tetrodotoxin, indicating its neural origin and consistent with the localization of 5-HT_3 receptors on sensory nerve endings in the intestinal tract (Fozard 1987). It must primarily involve the myenteric plexus as 1-phenylbiguanide had only a small effect in stripped ileum. Tetrodotoxin has also been shown to abolish the response to the 5-HT_3 agonist 2-methyl-5-HT in intact sheets of rat distal colon (Budhoo et al 1996). Tetrodotoxin had less effect on the actions of 5-HT and 5-methoxytryptamine which were only reduced by about 50% in intact preparations. In stripped preparations the tetrodotoxin-induced inhibition of the response to 5-HT was reduced to 25%. These findings indicate that neural mechanisms contribute to 5-HT-induced ileal secretion, but are not solely responsible. The effects of tetrodotoxin might vary with the species, the region of the intestine and the type of preparation studied. In rat jejunum tetrodotoxin reduces the response to 5-HT by 86% in intact sheets, but is without effect in stripped sheets (Hardcastle & Hardcastle 1996b). In stripped sheets of jejunum (Budhoo & Kellum 1994) or ileum (Burleigh & Borman 1993) from man tetrodotoxin is without effect, although in stripped sheets of guinea-pig ileum it causes significant inhibition (Cooke & Carey 1985; Baird & Cuthbert 1987). In rat colon tetrodotoxin inhibits the 5-HT response in intact preparations (Siriwardena et al 1991) but has no effect in stripped preparations (Zimmerman & Binder 1984; Bunce et al 1991; Siriwardena et al 1991). There might be a gradient of neural involvement in the action of 5-HT, with the greatest contribution observed in the jejunum. This effect is primarily mediated via the myenteric plexus as tetrodotoxin is much less effective in stripped preparations.

The fact that 5-methoxytryptamine induces a significantly greater response in stripped preparations suggests there might be an anti-secretory pathway present in intact sheets. The failure to observe this effect with 5-HT could be attributed to the activation of a 5-HT_3-mediated pro-secretory component which would be absent for 5-methoxytryptamine. The proposed anti-secretory component might be non-neural in origin as the residual response to 5-HT in the presence of tetrodotoxin is also greater in stripped preparations. A possible mediator of this anti-secretory action is nitric oxide (NO). Several studies have demonstrated that NO production is associated with enhanced basal absorption (Barry et al 1994; Rao et al 1994) and inhibition of the response to secretagogue challenge (Izzo et al 1994; Schirgi-Degen & Beubler 1995), although there are other reports suggesting that NO plays a pro-secretory role (Rhoads et al 1995; Kadowaki et al 1996). The involvement of NO therefore remains to be resolved.

The findings of this study implicate a number of different components in the secretory response of rat ileum to 5-HT. There is evidence for the involvement of both pro-secretory and anti-secretory pathways and the existence of a neural component to the response, and it is clear that 5-HT interacts with a number of different receptor subtypes in its activation of ileal secretion. The observed response is therefore likely to represent the sum of the effects of several different mechanisms.

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