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5-Hydroxytryptamine-induced Secretion by Rat Jejunum In-vitro Involves Several 5-Hydroxytryptamine Receptor Subtypes

J. HARDCASTLE AND P. T. HARDCASTLE

Department of Biomedical Science, University of Sheffield, UK

Abstract

The receptors contributing to 5-hydroxytryptamine (5-HT)-induced anion secretion by rat jejunum have been investigated by testing the effects of selective agonists and antagonists in-vitro using both intact and stripped intestinal sheets.

In both intact and stripped jejunum 5-HT and 5-methoxytryptamine, an agonist that lacks affinity for 5-HT_3 receptors, induced concentration-dependent increases in the short-circuit current (SCC), although 5-methoxytryptamine induced a smaller maximum response. In intact sheets 1-phenylbiguanide, a selective 5-HT_3 agonist, induced a response that was similar in magnitude to that of 5-methoxytryptamine, but in stripped preparations it had little effect. Tetrodotoxin inhibited the response of intact jejunum to 5-HT (by 86%) and 5-methoxytryptamine (by 85%) and abolished the response to 1-phenylbiguanide. In stripped sheets inhibition of the 5-HT response by tetrodotoxin was reduced to 27%. Desensitization to 1-phenylbiguanide reduced the response to 5-HT in intact but not stripped sheets whereas, in contrast, desensitization to 5-methoxytryptamine inhibited the 5-HT response in stripped sheets but was without effect in intact sheets. Mianserin, a 5-HT_1, 5-HT_2 and 5-HT_3 antagonist, and renzapride, a 5-HT_1 and 5-HT_3 antagonist, both reduced the maximum response to 5-HT, but 5-HTP-DP, a 5-HT_1 antagonist, was without effect. The 5-HT_3 antagonist granisetron reduced the response to 5-HT in intact, but not in stripped sheets. Tropisetron, a 5-HT_3 and 5-HT_4 antagonist, inhibited the response to 5-methoxytryptamine in both preparations, but did not alter the response to 5-HT.

It is concluded that 5-HT-induced jejunal secretion involves more than one 5-HT receptor subtype, with both neural and non-neural mechanisms contributing to the response.

5-Hydroxytryptamine (5-HT) is abundant throughout 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). That 5-HT induces a secretory response in the jejunum has been clearly established both in-vivo (Kisloff & Moore 1976; Zinner et al 1986; Beubler & Horina 1990; Beubler et al 1990, 1993; Hansen et al 1994a; Hardcastle et al 1994; Franks et al 1995, 1996) and in-vitro (Castro et al 1987; Urquhart et al 1988; Budhoo & Kellum 1994; Hansen 1994; Hansen et al 1994b, c; Hardcastle et al 1994; Kellum et al 1994; Budhoo et al 1996), although the mechanisms responsible are complex and have yet to be fully elucidated. There is evidence that 5-HT has several sites of action, with both neural and non-neural components contributing to the response (Castro et al 1987; Hansen 1994; Franks et al 1996; Hardcastle & Hardcastle 1997a, b). The situation is further complicated because the 5-HT receptor population comprises numerous subtypes (Bradley et al 1986; Hoyer & Schoeffter 1991; Hoyer et al 1994) and several of these have been implicated in the secretory response of the jejunum. These include 5-HT_1 (Castro et al 1987), 5-HT_2 (Beubler & Horina 1990; Beubler et al 1990, 1993; Hansen et al 1994c), 5-HT_3 (Beubler et al 1993; Hansen et al 1994c; Franks et al 1995) and 5-HT_4 (Budhoo & Kellum 1994; Hansen 1994; Kellum et al 1994; Budhoo et al 1996) receptors, although it has not generally proved possible to identify particular receptor subtypes with specific sites of action. It is however, considered that 5-HT_3 receptors in the intestinal tract are located on sensory neurons (Fozard 1987) and activate a cholinergic mechanism to stimulate secretion (Hendriks et al 1989;

Correspondence: J. Hardcastle, Department of Biomedical Science, University of Sheffield, Western Bank, Sheffield S10 2TN, UK.

Cooke et al 1991; Franks et al 1996). However, 5-HT_3 antagonists have little effect on the response to 5-HT, although they do cause marked inhibition of the secretory effects of selective 5-HT_3 agonists (Hardcastle & Hardcastle 1995). Moreover, 5-methoxytryptamine, a 5-HT agonist that lacks affinity for 5-HT_3 receptors, is also capable of producing a secretory response (Franks et al 1995; Hardcastle & Hardcastle 1995). These findings suggest that 5-HT_3 receptors cannot be the only subtype involved in the jejunal secretory response to 5-HT.

This study was designed to investigate further the mechanisms involved in 5-HT-induced secretion in rat jejunum by examining the effects of selective agonists and antagonists on both intact and stripped intestinal preparations.

Materials and Methods

Chemicals

5-Hydroxytryptamine creatinine sulphate, 5-methoxytryptamine, mianserin and tetrodotoxin were obtained from Sigma (Poole, UK) and 1-phenylbiguanide from Aldrich (Gillingham, Dorset, UK). Granisetron (BRL43694), 5-HTP-DP (*N*-acetyl-5-hydroxytryptophyl-5-hydroxytrytophan amide), renzapride (BRL24924) and tropisetron (ICS 205 930) were gifts from SmithKline Beecham Pharmaceuticals (Harlow, UK). All drugs were dissolved in 154 mM NaCl except 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 by intraperitoneal administration of sodium pentobarbitone (Sagatal, 60 mg kg^{-1}).

Measurement of trans-intestinal electrical activity across jejunal 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) proximal jejunum taken from the region immediately distal to the ligament of Treitz. 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 using 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 five 1-min basal readings 5-HT agonists were added to the serosal solution at the concentrations indicated. 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). Where 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 ± 1 s.e.m. of the number of observations indicated. Student's *t*-test, paired or unpaired as appropriate, was used to assess the significance of any differences observed. EC50 values (the concentration resulting in half the maximum effect) were calculated as geometric means (95% confidence limits) and statistical analysis was performed on log-transformed data. No estimate of EC50 was made when the maximum response was reduced.

Results

Jejunal response to 5-HT agonists

Intact sheets of rat jejunum generated a basal PD of 2·4 ± 0·1 mV, an SCC of 61·1 ± 1·5 $\mu A\,cm^{-2}$, serosa positive, and a tissue resistance of 41·2 ± 0·8 ohm cm^2 (n = 199). Stripped sheets (n = 184) had lower basal electrical activity (PD

1·5 ± 0·1 mV; $P < 0·001$; SCC 55·6 ± 2·1 $\mu A\,cm^{-2}$; $P < 0·05$; tissue resistance 28·6 ± 0·7 ohm cm^2; $P < 0·001$). 5-HT (100 μM) increased the SCC across jejunal sheets, with the response being greater in the intact preparation (intact 80·0 ± 6·5 $\mu A\,cm^{-2}$, n = 32; stripped 45·3 ± 4·1 $\mu A\,cm^{-2}$, n = 31; $P < 0·001$). 5-Methoxytryptamine (100 μM), an agonist that lacks affinity for 5-HT_3 receptors (Fozard 1985; Leff & Martin 1988; Craig et al 1990), also increased the SCC, the response again being greater in intact sheets (intact 37·0 ± 3·1 $\mu A\,cm^{-2}$, n = 27; stripped 15·7 ± 1·8 $\mu A\,cm^{-2}$, n = 24; $P < 0·001$), although in both preparations the increases in SCC induced by 5-methoxytryptamine were smaller than those observed with 5-HT ($P < 0·001$ in both cases). In intact sheets 1-phenylbiguanide (100 μM), a selective 5-HT_3 agonist (Hoyer et al 1994), induced an increase in SCC (46·2 ± 4·4 $\mu A\,cm^{-2}$; n = 26) that was similar to that obtained with 5-methoxytryptamine ($P > 0·05$), although smaller than the response to 5-HT ($P < 0·001$). In stripped sheets however, 1-phenylbiguanide caused only a very small increase in SCC (3·3 ± 1·3 $\mu A\,cm^{-2}$; n = 16), significantly lower than the effects of either 5-HT or 5-methoxytryptamine in this preparation ($P < 0·001$ for both). The actions of these agonists were concentration-dependent and in both intact and stripped preparations 5-HT again induced larger responses than either of the other two agonists (Figure 1, Table 1). The maximum response to 5-HT was greater in intact sheets ($P < 0·01$), although the stripped preparation was more sensitive, with a lower EC50 value ($P < 0·001$). Stripping the jejunum did not affect the response to 5-methoxytryptamine — maximum SCC changes and EC50 values were similar for intact and stripped sheets ($P > 0·05$ for both). In intact sheets the maximum increase in SCC obtained after cumulative addition of all three agonists was significantly lower than the change induced by the single application of the maximum concentration (100 μM). Cumulative addition of 5-HT produced a maximum SCC change that was 60% ($P < 0·001$) of that caused by a single application, whereas values for 5-methoxy-tryptamine and 1-phenylbiguanide were 68% ($P < 0·05$) and 39% ($P < 0·001$), respectively. In stripped sheets 5-HT was also less effective when added cumulatively, producing a maximum response that was 70% ($P < 0·001$) of that obtained with a single application. The reduced maximum response induced by the cumulative application of 5-HT has also been observed in rat colon (Bunce et al 1991) and indicates that some desensitization has occurred. The more pronounced discrepancy for 1-phenylbiguanide suggests that 5-HT_3 receptors are more susceptible to this phenomenon. In contrast, the maximum responses to 5-methoxytryptamine in stripped sheets were similar in the two protocols.

Effects of tetrodotoxin on the responses to 5-HT agonists

In intact sheets tetrodotoxin caused a 49% reduction in the basal SCC (control 60·2 ± 6·8 $\mu A\,cm^{-2}$; tetrodotoxin 31·0 ± 3·3 $\mu A\,cm^{-2}$; $P < 0·001$, n = 12).

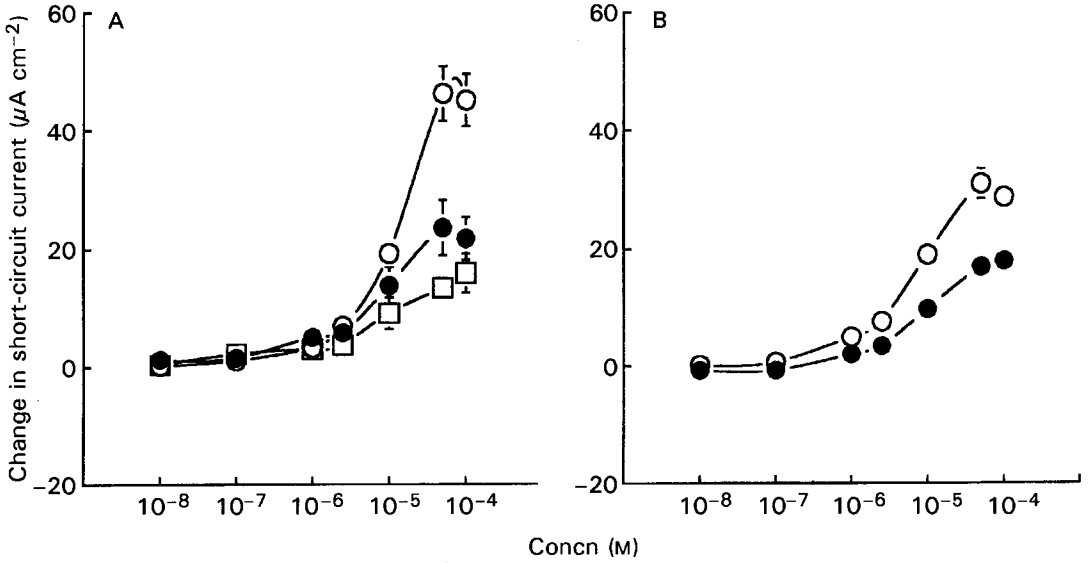

Figure 1. Concentration-dependence of 5-HT agonist action in A. intact and B. stripped sheets of rat jejunum. The increases in short-circuit current induced by cumulative additions of 5-HT (○; n = 62 (intact); n = 63 (stripped)), 5-methoxytryptamine (●; n = 17 (intact); (n = 27 (stripped)) and 1-phenylbiguanide (□; n = 11) are plotted as a function of log agonist concentration and each point represents the mean ± 1 s.e.m. of the number of observations indicated. Occasionally the error bars are smaller than the size of the symbols.

Table 1. Effects of 5-HT agonists on the short-circuit current generated by intact and stripped sheets of rat jejunum.

	n	Maximum increase in short-circuit current ($\mu A\,cm^{-2}$)	EC50 (μM)
Intact sheets			
5-HT	62	48·1 ± 4·6	14·3 (11·3–18·2)
5-Methoxytryptamine	17	25·1 ± 4·4*	11·0 (5·6–21·5)
1-Phenylbiguanide	11	18·1 ± 3·3†	10·8 (5·2–22·6)
Stripped sheets			
5-HT	63	31·6 ± 2·5	5·5 (4·5–6·6)
5-Methoxytryptamine	27	18·3 ± 2·4†	6·3 (4·7–8·6)

Cumulative concentration–response curves to 5-hydroxtryptamine, 5-methoxytryptamine and 1-phenylbiguanide were constructed. The increase in short-circuit current is expressed as the maximum response and each value represents the mean ± 1 s.e.m. of the number of observations indicated. EC50 values are geometric means (95% confidence limits). An unpaired *t*-test was used to assess the significance of any differences observed. * $P < 0.05$, † $P < 0.01$, significantly different from result for 5-hydroxtryptamine.

Table 2. Effects of serosal tetrodotoxin (10 μM) on the increase in short-circuit current induced by 100 μM 5-HT, 5-methoxytryptamine and 1-phenylbiguanide in intact and stripped sheets of rat jejunum.

	n	Increase in short-circuit current ($\mu A\,cm^{-2}$)	
		Control	With tetrodotoxin
Intact sheets			
5-HT	4	87·6 ± 15·1	11·3 ± 0·5‡
5-Methoxytryptamine	4	43·9 ± 7·7	0·0 ± 0·0‡
1-Phenylbiguanide	4	30·4 ± 12·8	2·5 ± 0·6†
Stripped sheets			
5-HT	6	39·5 ± 6·4	28·8 ± 5·6*

Each value is the mean ± 1 s.e.m. of the number of observations indicated. A paired *t*-test was used to assess the significance of tetrodotoxin action. * $P < 0.05$, † $P < 0.01$, ‡ $P < 0.001$, significantly different from the control result.

It also inhibited the responses to 5-HT (by 86%) and 5-methoxytryptamine (by 85%) and abolished the effects of 1-phenylbiguanide (Table 2). The increase in SCC induced by glucose was unaffected by tetrodotoxin (control 125·2 ± 14·0 $\mu A\,cm^{-2}$; tetrodotoxin 118·6 ± 14·1 $\mu A\,cm^{-2}$; $P > 0.05$, n = 12). In stripped sheets the basal SCC was not reduced by tetrodotoxin (control 52·8 ± 8·8 $\mu A\,cm^{-2}$; tetrodotoxin 45·2 ± 5·4 $\mu A\,cm^{-2}$; $P > 0.05$, n = 6) and the inhibition of the 5-HT response was reduced to 27% ($P < 0.05$). The glucose-dependent increase in SCC was again unaffected by tetrodotoxin (control 100·9 ± 7·2 $\mu A\,cm^{-2}$; tetrodotoxin 147·4 ± 38·2 $\mu A\,cm^{-2}$; $P > 0.05$, n = 6). The residual response to 5-HT in the presence of tetrodotoxin was greater in the stripped preparation (intact 11·3 ± 0·5 $\mu A\,cm^{-2}$, n = 4; stripped 28·8 ± 5·6 $\mu A\,cm^{-2}$, $P < 0.05$, n = 6) although in the absence of the neurotoxin it was intact sheets that generated the larger 5-HT response ($P < 0.001$).

Effects of desensitization

In-vitro desensitization of 5-HT receptors by repeated application of agonist leads to loss of the response (Hubel 1984; Cooke & Carey 1985; Castro et al 1987; Hardcastle et al 1994). A second application of either 1-phenylbiguanide or 5-methoxytryptamine failed to elicit an increase in SCC in either intact or stripped sheets (Table 3). Desensitization to 1-phenylbiguanide reduced the response to 5-HT in intact sheets, but was without effect in stripped sheets (Table 4). In contrast, desensitization to 5-methoxytryptamine abolished the 5-HT response in the stripped preparation, but had no effect in intact jejunum (Table 4). Previous exposure to a combination of both 1-phenylbiguanide and 5-methoxytryptamine reduced the 5-HT-

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

	n	Increase in short-circuit current ($\mu A\,cm^{-2}$)	
		First application	Second application
Intact sheets			
1-Phenylbiguanide	11	55·3 ± 8·0	−1·3 ± 0·5†
5-Methoxytryptamine	12	28·1 ± 4·8	−0·2 ± 0·8†
1-Phenylbiguanide + 5-methoxytryptamine	8	48·8 ± 8·0	−1·4 ± 0·5†
Stripped sheets			
1-Phenylbiguanide	7	6·0 ± 2·1	−2·7 ± 1·3*
5-Methoxytryptamine	8	27·6 ± 4·3	−2·3 ± 0·9†
1-Phenylbiguanide + 5-methoxytryptamine	8	40·0 ± 3·9	−3·5 ± 1·6†

Test sheets received two consecutive applications of agonist (both 100 μM) at 10-min intervals while control sheets received equivalent volumes of vehicle (2% v/v). The increases in short-circuit current induced by 5-hydroxtryptamine agonists are given as mean values ± 1 s.e.m. of the number of observations indicated. A paired t-test was use to compare the first and second responses. * $P < 0.01$, † $P < 0.001$, significantly different from the first response. Addition of vehicle had no effect on the short-circuit current ($P > 0.05$ in all cases).

Table 4. Responses of intact and stripped sheets of rat jejunum to 100 μM 5-HT after previous exposure to two consecutive applications of 1-phenylbiguanide, 5-methoxytryptamine or 1-phenylbiguanide plus 5-methoxytryptamine (100 μM in each case).

	n	Increase in short-circuit current ($\mu A\,cm^{-2}$)	
		Control	Test
Intact sheets			
1-Phenylbiguanide	8	89·4 ± 13·7	58·8 ± 9·7*
5-Methoxytryptamine	8	74·6 ± 8·3	73·1 ± 12·3
1-Phenylbiguanide + 5-methoxytryptamine	8	85·5 ± 12·2	14·7 ± 3·0‡
Stripped sheets			
1-Phenylbiguanide	7	37·6 ± 4·9	43·3 ± 10·0
5-Methoxytryptamine	8	35·2 ± 5·4	1·73 ± 1·7‡
1-Phenylbiguanide + 5-methoxytryptamine	8	48·8 ± 5·2	−0·7 ± 0·7‡

Control sheets received an equivalent volume of vehicle (2% v/v). Each value is the mean ± 1 s.e.m. of the number of observations indicated. A paired t-test was used to compare the responses in control and test sheets. * $P < 0.05$, ‡ $P < 0.001$, significantly different from control result.

induced increase in SCC in intact sheets and eliminated it in stripped sheets (Table 4).

Effects of 5-HT antagonists

Mianserin, an antagonist at 5-HT_1, 5-HT_2 and 5-HT_3 receptors (Hoyer & Schoeffter 1991; Wood et al 1993), inhibited the maximum response to 5-HT in both intact and stripped preparations (Table 5). However, in stripped jejunum at the same concentration it failed to influence either the maximum response (control 18·3 ± 3·3 $\mu A\,cm^{-2}$; mianserin 15·5 ± 3·2 $\mu A\,cm^{-2}$, n = 5; $P > 0.05$) or the EC50 (control 5·9 (2·9–12·4) μM; mianserin 6·3 (2·6–15·0) μM, n = 5; $P > 0.05$) induced by 5-methoxytryptamine.

Renzapride is a substituted benzamide which acts as an antagonist at 5-HT_{1P} (Gershon et al 1990) and 5-HT_3 (Hoyer & Schoeffter 1991) receptors, but also has agonist activity at 5-HT_4 receptors (Hoyer & Schoeffter 1991). It caused a concentration-dependent inhibition of the maximum response to 5-HT in both intact and stripped preparations (Figure 2, Table 5). In contrast, 5-HTP-DP, which is also an antagonist at 5-HT_{1P} receptors (Gershon et al 1990), failed to influence 5-HT action in either intact or stripped jejunum (Table 5).

In intact sheets the 5-HT_3 antagonist granisetron (1·4 μM; Sanger & Nelson (1989)) reduced the maximum SCC change induced by both 5-HT (control 87·1 ± 16·3 $\mu A\,cm^{-2}$; granisetron 16·4 ± 8·2 $\mu A\,cm^{-2}$, n = 5; $P < 0.01$) and 1-phenylbiguanide (control 17·2 ± 3·2 $\mu A\,cm^{-2}$; granisetron 4·2 ± 1·7 $\mu A\,cm^{-2}$, n = 7; $P < 0.01$), but in stripped sheets it had no effect on either the maximum response to 5-HT (control 32·0 ± 7·7 $\mu A\,cm^{-2}$; granisetron 27·1 ± 5·5 $\mu A\,cm^{-2}$, n = 6; $P > 0.05$) or the EC50 value (control 7·6 (3·5–16·7) μM; granisetron 3·2 (1·0–10·8) μM, n = 6; $P > 0.05$).

Table 5. Effects of 5-HT antagonists on the responses of intact and stripped sheets of rat jejunum to 5-HT.

	n	Maximum increase in short-circuit current ($\mu A\,cm^{-2}$)	EC50 (μM)
Intact sheets			
5-HT	6	43·6 ± 12·7	21·6 (10·0–47·4)
+ 10 μM mianserin	6	8·8 ± 2·2*	–
5-HT	8	50·2 ± 11·1	10·5 (7·4–15·0)
+ 10 μM *N*-acetyl-5-hydroxytryptophyl-5-hydroxytrytophan amide	8	45·6 ± 10·7	11·4 (7·1–18·5)
5-HT	8	36·0 ± 10·2	13·0 (7·0–24·1)
+ 1·4 μM renzapride	8	26·8 ± 7·9*	–
5-HT	8	36·5 ± 7·0	14·8 (10·8–20·4)
+ 7·0 μM renzapride	8	9·7 ± 4·5†	–
Stripped sheets			
5-HT	6	35·1 ± 10·1	11·9 (6·9–20·3)
+ 10 μM mianserin	6	9·8 ± 2·1*	–
5-HT	8	28·2 ± 4·3	6·5 (4·3–9·8)
+ 10 μM *N*-acetyl-5-hydroxytryptophyl-5-hydroxytrytophan amide	8	35·4 ± 5·0	10·1 (5·5–18·6)
5-HT	8	40·5 ± 4·0	9·1 (5·6–14·7)
+ 1·4 μM renzapride	8	16·8 ± 5·7†	–
5-HT	8	29·3 ± 7·2	7·5 (4·2–13·5)
+ 7·0 μM renzapride	8	8·3 ± 2·7*	–

Antagonists were present in the serosal solution of test sheets; control sheets received an equivalent volume of vehicle. The increase in short-circuit current is expressed as the maximum response and each value is the mean ± 1 s.e.m. of the number of observations indicated. EC50 values are geometric means (95% confidence limits). A paired *t*-test was used to assess the significance of antagonist action. * $P < 0.05$, † $P < 0.01$, significantly different from result without antagonist.

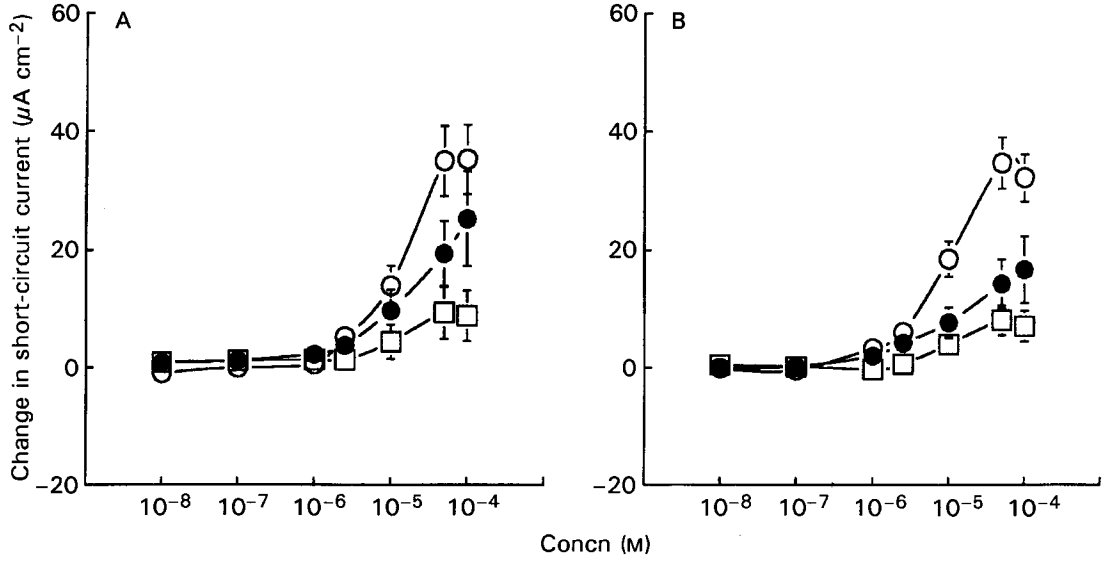

Figure 2. Effects of renzapride (● 1.4 or □ 7 μM) in the serosal solution on the responses of A. intact and B. stripped sheets of rat jejunum to 5-HT. The increases in short-circuit current induced by cumulative additions of 5-HT are plotted as a function of log agonist concentration and each point represents the mean ± 1 s.e.m. of eight observations. For each preparation control responses to 5-HT have been combined (○; n = 16). Occasionally the error bars are smaller than the size of the symbols.

Tropisetron (5 μM), a 5-HT_3 and 5-HT_4 antagonist (Buchheit et al 1992), neither reduced the maximum response to 5-HT nor increased the EC50 in intact or stripped jejunum ($P > 0.05$ in all cases), but it did inhibit the response to 5-methoxytryptamine. In intact preparations the maximum response to 5-methoxytryptamine was reduced from 28·0 ± 4·1 to 4·9 ± 2·1 $\mu A\,cm^{-2}$, n = 6 ($P < 0.01$) whereas in stripped preparations the maximum response remained unchanged (control 11·3 ± 3·9 $\mu A\,cm^{-2}$; tropisetron 8·6 ± 2·4 $\mu A\,cm^{-2}$, n = 7; $P > 0.05$), but the EC50 value increased from 4·7 (2·3–9·5) to 25·7 (17·6–34·5) μM ($P < 0.01$).

None of the antagonists tested affected the basal SCC nor the increase associated with active sodium-dependent glucose transport in either intact or stripped preparations ($P > 0.05$ in all cases),

except for tropisetron, which reduced the basal SCC in intact (from $58{\cdot}3 \pm 2{\cdot}9$ to $45{\cdot}8 \pm 2{\cdot}5\ \mu A\,cm^{-2}$, $n = 13$; $P < 0{\cdot}01$) but not in stripped sheets.

Discussion

The jejunal response to 5-HT was mimicked in intact sheets of jejunum by both 1-phenylbiguanide and 5-methoxytryptamine, although neither agonist produced a maximum response that was as large as that obtained with 5-HT itself. Because 1-phenylbiguanide is a selective 5-HT_3 agonist whereas 5-methoxytryptamine lacks affinity for this receptor subtype, it is evident that more than one 5-HT receptor subtype contributes to the secretory response of rat jejunum. The action of 1-phenylbiguanide was abolished by the neurotoxin tetrodotoxin, which is consistent with the involvement of neural 5-HT_3 receptors (Hendriks et al 1989; Cooke et al 1991; Franks et al 1996). 5-HT_3 agonist action has also been shown to be entirely neurally-mediated in both rat ileum (Hardcastle & Hardcastle 1997a) and colon (Siriwardena et al 1993). As 1-phenylbiguanide had little effect in stripped jejunum it seems that 5-HT_3-mediated secretion requires the presence of the myenteric plexus. Tetrodotoxin inhibited the response of intact jejunal sheets to 5-HT by 86%, confirming previous observations in intact preparations (Castro et al 1987; Hardcastle & Hardcastle 1997b) and indicating that neural mechanisms also play a major role in the response to 5-HT itself in this region of the rat intestinal tract. This cannot be solely attributed to an action mediated by 5-HT_3 receptors as the response to 5-methoxytryptamine was reduced to a comparable extent, a finding similar to that reported for stripped sheets of pig jejunum (Hansen 1994). Thus the secretory response activated by receptors other than 5-HT_3 must also involve neural pathways. The myenteric plexus seems to be a major component of the neural mechanisms contributing to 5-HT-induced jejunal secretion because in stripped sheets tetrodotoxin only caused a 27% reduction in the response to 5-HT. In stripped preparations of jejunum from both man (Budhoo & Kellum 1994; Budhoo et al 1996) and pig (Hansen et al 1994b) no inhibition of the 5-HT-induced rise in SCC by tetrodotoxin could be detected. Thus the submucosal and mucosal plexuses can only make a minor contribution to 5-HT action in the jejunum.

The residual response to 5-HT in the presence of tetrodotoxin was greater in the stripped preparation, despite the larger response from the intact sheets in the absence of the neurotoxin. This suggests that a non-neural, i.e. tetrodotoxin-insensitive, inhibitory mechanism is removed with the muscle layers and myenteric plexus. One possible candidate for this role is nitric oxide which is produced by the enterocytes (Tepperman et al 1993), where its release is unlikely to be affected by tetrodotoxin, and which has been reported to have a pro-absorptive action in rat jejunum (Schirgi-Degen & Beubler 1995).

The involvement of different 5-HT receptor subtypes was examined in experiments in which the 5-HT response was tested after desensitization to either 1-phenylbiguanide or 5-methoxytryptamine, an approach that has been previously used in the identification of the 5-HT receptors involved in rat ileal (Hardcastle & Hardcastle 1997a) and colonic (Leung et al 1995) secretion and guinea-pig ileal motility (Craig et al 1990). Desensitization to 1-phenylbiguanide inhibited the 5-HT-induced rise in SCC in intact, but not stripped sheets of jejunum, consistent with 5-HT_3 action requiring the presence of the myenteric plexus. In contrast, desensitization to 5-methoxytryptamine had no effect in intact sheets, but induced inhibition in stripped sheets. The lack of effect in intact sheets might seem surprising because receptors other than 5-HT_3 can activate secretion in this preparation, as is evidenced by the ability of 5-methoxytryptamine to increase the SCC. It is possible however, that desensitization of the receptors activated by 5-methoxytryptamine enables increased access of 5-HT to 5-HT_3 receptors and hence no diminution of the overall response. Desensitization to a combination of both 1-phenylbiguanide and 5-methoxytryptamine abolished 5-HT-induced secretion in stripped sheets and caused 83% inhibition in intact sheets. The generation of a small residual response in intact sheets suggests the existence of 5-HT receptors not activated by either agonist. The data from these experiments add further support to the view that several different 5-HT receptor subtypes contribute to 5-HT-induced intestinal secretion.

Several 5-HT antagonists were tested for their ability to inhibit 5-HT-induced jejunal secretion. Renzapride, an agonist at 5-HT_4 receptors and an antagonist at 5-HT_{1P} and 5-HT_3 receptors, inhibited the response to 5-HT in both intact and stripped preparations. Similar effects have been reported in pig jejunum (Hansen et al 1994c) and guinea-pig colon (Sidhu & Cooke 1995). This inhibition by renzapride cannot be attributed to its action at 5-HT_{1P} receptors because the 5-HT_{1P} antagonist 5-HTP-DP was without effect, confirming observations in the jejunum in man (Budhoo & Kellum

1994; Budhoo et al 1996) and in rat colon (Siriwardena & Kellum 1993). In intact sheets the ability of renzapride to block 5-HT_3 receptors could contribute to its inhibitory actions, because granisetron, a selective 5-HT_3 antagonist, also reduced the maximum response to 5-HT. This explanation cannot account for the effect of renzapride in stripped preparations because there is no evidence here for 5-HT_3 receptor action, as granisetron failed to inhibit the 5-HT response and 1-phenylbiguanide was unable to increase the SCC. It is possible that in stripped sheets desensitization of 5-HT_4 receptors by renzapride could have occurred and this might have influenced the actions of 5-HT. The involvement of 5-HT_4 receptors in the secretory response is suggested by the inhibition by tropisetron, a 5-HT_3 and 5-HT_4 antagonist, of the response to 5-methoxytryptamine, an agonist lacking affinity for 5-HT_3 receptors. However, tropisetron failed to influence the effects of 5-HT, a finding also reported for stripped sheets of pig jejunum (Hansen 1994). Interestingly, tropisetron did cause a small (21%), but significant reduction in the basal SCC, suggesting that tropisetron-sensitive sites mediate an action of endogenous 5-HT on jejunal ion transport. Mianserin, an antagonist at 5-HT_1, 5-HT_2 and 5-HT_3 receptors, inhibited the maximum response to 5-HT in both intact and stripped preparations, although it was without effect on the response to 5-methoxytryptamine. 5-HT_3 receptor antagonism could explain its actions in intact sheets, but in stripped sheets its ability to block 5-HT_1 and 5-HT_2 receptors might be more important. The selective 5-HT_2 antagonist ketanserin has been shown to inhibit the 5-HT-induced increase in SCC in stripped sheets of pig jejunum (Hansen et al 1994c), although in the jejunum in man it was without effect (Budhoo & Kellum 1994; Budhoo et al 1996), suggesting species differences can occur. The actions of the antagonists tested clearly point to the involvement of 5-HT_3 and 5-HT_4 receptors in the secretory response to 5-HT. Any possible contribution of 5-HT_1 and 5-HT_2 receptors remains to be resolved.

The findings of this study indicate that several different components are involved in the secretory response of rat jejunum to 5-HT. Neural mechanisms make a major contribution to 5-HT action and there is evidence for both pro-secretory and anti-secretory pathways. In addition, it is clear that 5-HT can interact with several different receptor subtypes in its activation of jejunal secretion. The observed response is therefore likely to represent the sum of the effects of several different mechanisms.

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