Comparison of the Secretory Actions of 5-Hydroxytryptamine in the Proximal and Distal Colon of the Rat

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Abstract

The ability of 5-hydroxytryptamine (5-HT) to induce a secretory response in rat proximal and distal colon was examined both in-vivo and in-vitro by measuring transintestinal electrical activity.

In-vivo 5-HT caused a dose-dependent increase in the potential difference (PD) in both regions of the colon (maximum PD change = 7.2 ± 0.5 (n = 17) mV in proximal colon and 9.2 ± 0.7 (n = 17) mV in distal colon), an effect that was also observed in stripped (outer muscle layers removed) colonic sheets where the PD change was found to result from a rise in short-circuit current (SCC, maximum change = 150 ± 24 (n = 15) $\mu A\,cm^{-2}$ in proximal colon and 126 ± 10 (n = 19) $\mu A\,cm^{-2}$ in distal colon).

The effects of 2-methyl-5-hydroxytryptamine (2-Me-5-HT), a relatively selective agonist at 5-HT_3 receptors, and 5-methoxytryptamine (5-MT), an agonist at all 5-HT receptors except 5-HT_3, were also tested, their specificity of action being confirmed by their actions on cardiovascular function in-vivo. 2-Me-5-HT produced a similar response to 5-HT in proximal colon, but was less effective in the distal region, particularly in-vitro where it failed to induce any significant change in electrical activity. In contrast, 5-MT was more effective in the distal colon. Frusemide (10^{-3} M) inhibited the rise in SCC induced by both 2-Me-5-HT and 5-MT, indicating that, like 5-HT, these agonists stimulated electrogenic Cl^- secretion. The 5-HT_3 antagonist granisetron abolished the effects of 2-Me-5-HT, both in-vivo ($8.6 \times 10^{-8}\,mol\,kg^{-1}$) and in-vitro ($1.4 \times 10^{-6}$ M, 1.4×10^{-4} M), but only caused a slight inhibition of the response to 5-HT in-vivo and no inhibition at all in stripped colonic sheets.

It is concluded that although 5-HT induces a secretory response in both proximal and distal colon, the mechanisms responsible differ, with 5-HT_3 receptors making a greater contribution in the proximal region.

The ability of 5-hydroxytryptamine (5-HT) to produce a secretory response in the mammalian colon is well-established (Zimmerman & Binder 1984; Bunce et al 1991; Cooke et al 1991; Siriwardena et al 1991). This response results from a stimulation of electrogenic Cl^- secretion together with an inhibition of electroneutral NaCl absorption, changes that lead to a prosecretory alteration in net fluid movement across the colon (Zimmerman & Binder 1984). A large number of 5-HT receptor subtypes have now been described (Fozard 1987; Tally 1992), but a clear picture concerning those responsible for intestinal secretion has yet to emerge. One group has concluded that the receptor mechanism responsible for 5-HT-induced secretion by rat distal colon cannot be identified as 5-HT_1-like, 5-HT_2 or 5-HT_3 (Bunce et al 1991) while another group, using the same preparation, report evidence for the involvement of 5-HT_2 receptors (Siriwardena et al 1991). The situation is further complicated by the segmental heterogeneity that exists along the length of the colon (Fromm & Hegel 1978; Nobles et al 1991). Under basal conditions the predominant transport activity of the proximal colon is electrogenic Cl^- secretion, while in the distal colon it is electroneutral NaCl absorption. These differences account for the larger basal short-circuit current (SCC) observed in the proximal region. In addition, there are differences in tissue resistance, which is greater in the distal colon. The

present investigation was designed to determine whether these functional differences between proximal and distal regions of the colon extended to the 5-HT-induced stimulation of electrogenic Cl^- secretion and to examine the possible involvement of 5-HT_3 receptors in the responses using selective agonists and antagonists.

Materials and Methods

Animals

Experiments were carried out 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 in-vivo

The transintestinal potential difference (PD) was measured across 2·5 cm segments of proximal and distal colon. Each segment was tied at the distal end and filled with 154 mM NaCl through a cannula inserted at the proximal end. The PD across each loop was measured between two salt bridge electrodes, one in contact with the luminal fluid and the other, via a wick electrode, with the peritoneal cavity. Electrodes were connected via calomel half-cells to two differential input electrometers whose outputs were displayed on a 2-channel chart recorder (Linseis L6512). Drugs were administered through a cannula in the femoral vein and each dose (in 0·1 mL) was washed in with 0·2 mL

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154 mM NaCl. The response to an agonist was taken as the difference between the maximum PD achieved after each dose and the value immediately before its addition.

During these experiments arterial blood pressure was monitored at the left carotid artery via a saline/heparin-filled cannula connected to a pressure transducer (Druck Ltd PDCR75). This was linked to a preamplifier (Lectromed type 5241) and a visual display obtained on a 2-channel chart recorder (Lectromed Multitrace 2). The blood pressure signal was fed into a ratemeter (Lectromed type 5250) to provide a continuous display of the heart rate integrated over a 1-s time base.

Body temperature was maintained at 37°C by a homeothermic blanket system (Harvard Apparatus Ltd).

In each preparation a 5-HT dose-response curve was constructed before the effects of other agonists or granisetron were tested.

Measurement of transintestinal electrical activity across colonic sheets

The PD, SCC and tissue resistance were measured across stripped sheets of colon from which the outer muscle layers and myenteric plexus had been removed. The entire colon was removed from the anaesthetized rat and, after stripping, the proximal sheet was prepared from the 2 cm immediately adjacent to the caecum and the distal colon from the 2 cm adjacent to the rectum. 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 gassed with 95% O_2–5% CO_2. The serosal fluid contained 10 mM glucose and the mucosal fluid 10 mM mannitol and each had a volume of 5 mL. The PD was measured using salt-bridge electrodes connected via calomel half-cells to a differential input electrometer with output to a Vitatron 2-channel chart recorder (MSE Scientific Instruments, 2001 series). 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 SCC generated by the sheets was calculated from PD and resistance measurements using Ohm's law.

Cumulative concentration–response curves to agonists were constructed by making the next addition 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 were investigated, the drug was added to the serosal solution at the concentration indicated 10 min before the first application of agonist.

Expression of results

Results are expressed as mean values ± s.e.m. of the number of observations indicated. In the in-vivo experiments, a paired t-test was used to assess the significance of the difference between control and test data. An unpaired t-test was used to compare proximal and distal colonic sheets. EC50 values were calculated as geometric means (95% confidence limits) and statistical analysis was performed on log transformed data. In some in-vivo experiments a maximum response could not be demonstrated, as high doses of 5-HT caused such a profound fall in blood pressure that the animal died. The greatest change obtained is, therefore, taken as the maximum response.

Chemicals

Frusemide, 5-hydroxytryptamine creatinine sulphate and 5-methoxytryptamine (5-MT) were obtained from Sigma Chemical Co. Ltd, Poole, UK, and dimethylsulphoxide (DMSO) from BDH Chemicals Ltd, Poole, UK. 2-Methyl-5-hydroxytryptamine (2-Me-5-HT) and granisetron (BRL43694) were gifts from SmithKline Beecham Pharmaceuticals, Harlow, UK.

Results

5-HT action in-vivo

The basal PD was 11·5 ± 0·8 mV in the proximal colon and 9·8 ± 0·9 mV in the distal colon (n = 17), serosa positive in both cases. 5-HT caused a dose-dependent rise in the PD across both regions (Fig. 1), with the maximum response being significantly greater in the distal colon (proximal: 7·2 ± 0·5 mV; distal: 9·2 ± 0·7 mV, n = 17, P < 0·05). The proximal colon was more sensitive to the action of 5-HT
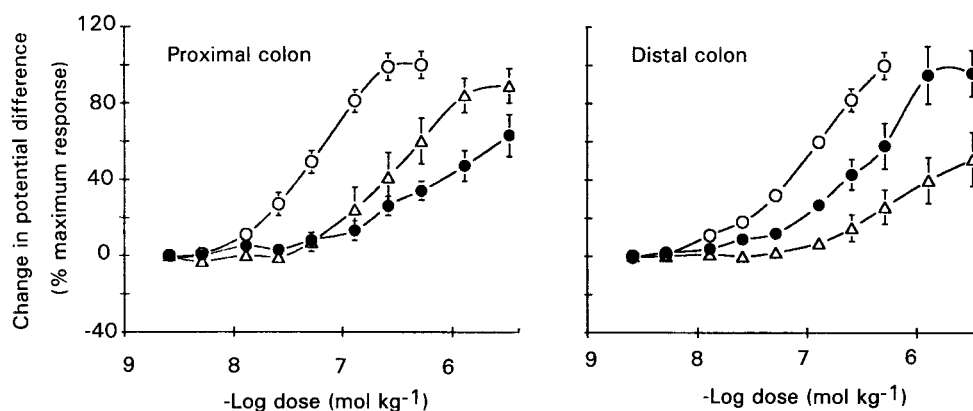

FIG. 1. Effects of 5-HT, 2-Me-5-HT and 5-MT on the PD across rat proximal and distal colon in-vivo. The changes in PD induced by 5-HT (○, n = 17, combined control data), 2-Me-5-HT (△, n = 6) and 5-MT (●, n = 5) are expressed as % maximum response to 5-HT with the 100% value being 7·2 ± 0·5 mV in the proximal colon and 9·2 ± 0·7 mV in the distal colon. The changes in PD are plotted as a function of log agonist dose and each point represents the mean ± s.e.m. of the number of observations indicated. In some cases the size of the error bar is less than the size of the symbol.

Table 1. Effects of 5-HT, 2-Me-5-HT and 5-MT on cardiovascular function in the rat. The effects of 5-HT in the presence of granisetron $(8\cdot6 \times 10^{-8}\,nmol\,kg^{-1})$ are also shown.

Experiment	n	Change in heart rate		Change in systolic blood pressure		Change in diastolic blood pressure	
		Max (beats min^{-1})	EC50 (μmol kg^{-1})	Max (mmHg)	EC50 (μmol kg^{-1})	Max (mmHg)	EC50 (μmol kg^{-1})
5-HT	17	277 ± 20	0·14 (0·099–0·19)	63 ± 4	0·28 (0·15–0·55)	62 ± 2	0·023 (0·018–0·029)
2-Me-5-HT	6	118 ± 28**	0·26 (0·084–0·80)	11 ± 5**	—	13 ± 2***	—
5-HT + granisetron	6	28 ± 19**	—	78 ± 5**	0·17 (0·12–0·24)	53 ± 2*	0·026 (0·016–0·043)
5-MT	5	62 ± 28***	—	76 ± 6	0·40 (0·22–0·72)	54 ± 3	0·037 (0·014–0·096)

The initial fall in heart rate, the rise in systolic pressure and the prolonged fall in diastolic pressure are expressed in terms of the maximum response (mean ± s.e.m.) and the EC50 (geometric mean, 95% confidence limits) of the number of observations indicated. *$P < 0\cdot05$, **$P < 0\cdot01$, ***$P < 0\cdot001$ compared with the control 5-HT values.

with an EC50 value of $4\cdot6 \times 10^{-8}$ $(3\cdot3–6\cdot2 \times 10^{-8})\,mol\,kg^{-1}$ compared with a value of $8\cdot7 \times 10^{-8}$ $(7\cdot2 \times 10^{-8}–1\cdot1 \times 10^{-7})\,mol\,kg^{-1}$ in the distal colon (n = 17, $P < 0\cdot001$).

Cardiovascular function was monitored as heart rate, together with systolic and diastolic pressures. Basal values were 412 ± 9 beats min^{-1}, 147 ± 4 mmHg and 113 ± 4 mmHg, respectively (n = 17) and did not change significantly over the period of the experiment. 5-HT caused a dose–dependent triphasic change in cardiovascular function (Table 1)—an initial transient bradycardia and fall in blood pressure (the Bezold-Jarisch reflex) mediated by 5-HT$_3$ receptors, followed by a rise in systolic pressure mediated by 5-HT$_2$ receptors, and finally a prolonged fall in diastolic pressure mediated by 5-HT$_1$-like receptors (Kalkman et al 1984). These responses can be used as an index of the selectivity of agonist and antagonist action.

2-Me-5-HT, a 5-HT$_3$ agonist (Richardson et al 1985), caused a transient bradycardia and fall in blood pressure with no pressor or prolonged depressor phase, confirming that it was acting selectively at 5-HT$_3$ receptors over the dose range tested (Table 1). The 5-HT$_3$ antagonist granisetron (Sanger & Nelson 1989), at a dose of $8\cdot6 \times 10^{-8}\,mol\,kg^{-1}$, abolished the initial cardiovascular response to 5-HT without affecting the pressor or prolonged depressor

phases (Table 1). It also inhibited the cardiovascular action of 2-Me-5-HT, reducing the heart rate response to the highest dose of agonist tested $(3\cdot3 \times 10^{-6}\,mol\,kg^{-1})$ from 118 ± 28 (n = 6) to 48 ± 25 (n = 6) beats min^{-1}, a value that did not differ significantly from zero $(P > 0\cdot05)$.

5-MT did not elicit the Bezold-Jarisch reflex, but it did produce pressor and prolonged depressor responses (Table 1), confirming its ability to activate 5-HT$_1$-like and 5-HT$_2$, but not 5-HT$_3$, receptors (Fozard 1985; Leff & Martin 1988; Craig et al 1990).

2-Me-5-HT caused a dose-dependent rise in the PD across both proximal and distal colon, with EC50 values that were greater than those observed with 5-HT, but which did not differ in the two regions $(P > 0\cdot05$, Fig. 1, Table 2). In the proximal colon the maximum response to 2-Me-5-HT did not differ from that obtained with 5-HT, but in the distal colon it was significantly lower (Table 2).

Granisetron $(8\cdot6 \times 10^{-8}\,mol\,kg^{-1})$ abolished the PD response to 2-Me-5-HT in both proximal and distal regions of the colon, but it had only a small effect on the actions of 5-HT, reducing the maximum PD change without altering the EC50 value (Fig. 2, Table 2). Increasing the dose of granisetron to $8\cdot6 \times 10^{-7}\,mol\,kg^{-1}$ did not cause any further inhibition of either the PD or heart rate responses to 5-HT

Table 2. Effects of 5-HT, 2-Me-5-HT and 5-MT on the PD across rat proximal and distal colon in-vivo. The effects of 5-HT in the presence of granisetron $(8\cdot6 \times 10^{-8}\,mol\,kg^{-1})$ are also shown.

Experiment	n	Proximal colon		Distal colon	
		ΔPD$_{max}$ (mV)	EC50 (μmol kg^{-1})	ΔPD$_{max}$ (mV)	EC50 (μmol kg^{-1})
5-HT	6	7·5 ± 0·8	0·043 (0·025–0·074)	9·1 ± 1·3	0·087 (0·065–0·12)
2-Me-5-HT		6·8 ± 0·7	0·33*** (0·16–0·71)	5·4 ± 1·3*	0·29** (0·16–0·54)
5-HT	6	7·6 ± 0·7	0·05 (0·022–0·11)	7·6 ± 0·7	0·079 (0·046–0·14)
5-HT + granisetron		5·8 ± 0·9*	0·079 (0·038–0·17)	5·5 ± 0·8**	0·081 (0·050–0·13)
5-HT	5	6·3 ± 0·9	0·044 (0·025–0·079)	11·3 ± 1·2	0·099 (0·067–0·15)
5-MT		4·6 ± 0·8*	0·48 (0·19–1·2)	11·7 ± 1·6	0·39** (0·26–0·58)

The rise in PD is expressed in terms of the maximum response (mean ± s.e.m.) and the EC50 (geometric mean, 95% confidence limits) of the number of observations indicated. *$P < 0\cdot05$, **$P < 0\cdot01$, ***$P < 0\cdot001$ compared with the control value.
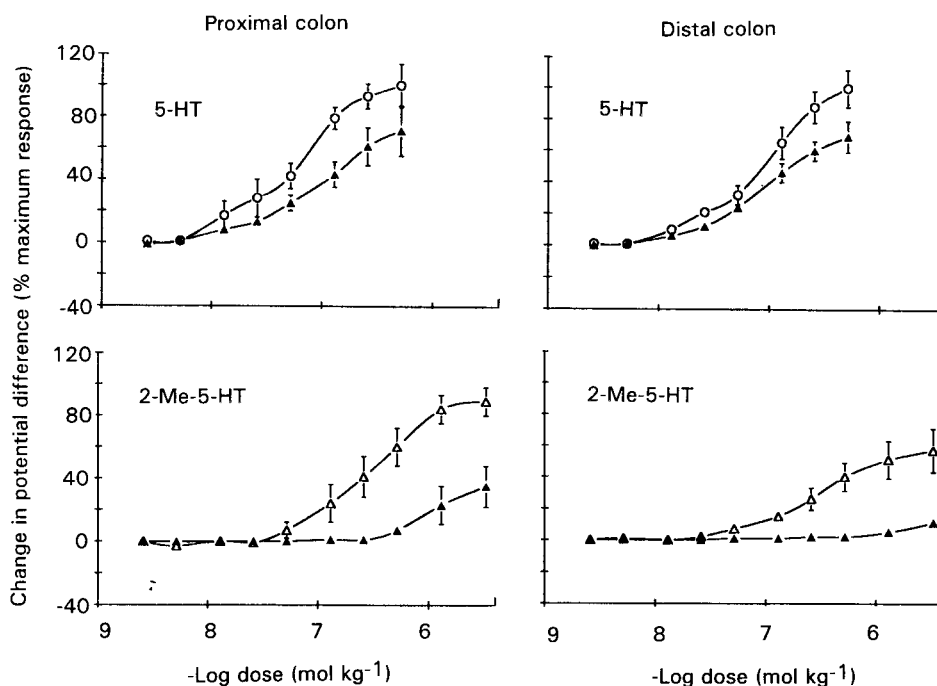
FIG. 2. Effect of granisetron on the responses of rat proximal and distal colon to 5-HT and 2-Me-5-HT in-vivo. The changes in PD induced by 5-HT (○, n = 6) or 2-Me-5-HT (△, n = 6) in the absence or presence of granisetron (▲, 8.6×10^{-8} mol kg^{-1}) are expressed as % maximum response to 5-HT with 100% values in the proximal and distal colon being 7.5 ± 0.8 and 9.1 ± 1.3 mV, respectively, for the 5-HT experiments and 7.6 ± 1.1 and 7.8 ± 0.9 mV, respectively, for the 2-Me-5-HT experiments. The changes in PD are plotted as a function of log agonist dose and each point represents the mean ± s.e.m. of the number of observations indicated. In some cases the size of the error bar is less than the size of the symbol.

($P > 0.05$ in all cases), nor did it affect the pressor and prolonged depressor effects of the amine.

5-MT increased the PD across proximal and distal colon in a dose-dependent fashion, again with EC50 values that were greater than those obtained with 5-HT, but not different in the two regions (Fig. 1, Table 2, $P > 0.05$). In contrast to 2-Me-5-HT, 5-MT caused a similar maximum PD change to 5-HT in the distal colon, but a lower value in the proximal colon (Table 2).

5-HT action in colonic sheets

Under control conditions, the proximal colon generated a basal PD of 8.1 ± 0.6 mV, a SCC of $146 \pm 10 \mu$A cm^{-2} and

had a tissue resistance of 57 ± 3 ohm cm^2 (n = 35). The distal colon (n = 43) generated a similar basal PD (6.9 ± 0.5 mV, $P > 0.05$), but the SCC was significantly lower ($88 \pm 7 \mu$A cm^{-2}, $P < 0.001$) while the tissue resistance was significantly higher (85 ± 5 ohm cm^2, $P < 0.001$).

5-HT caused a concentration-dependent rise in SCC in both proximal and distal colonic sheets (Fig. 3, Table 3), with more variability in the proximal segment. The maximum responses were similar in the two regions ($P > 0.05$), while the EC50 value was lower in the distal segment ($P < 0.05$). In the proximal colon 2-Me-5-HT produced similar changes to those observed with 5-HT, but in the distal colon it was without significant effect
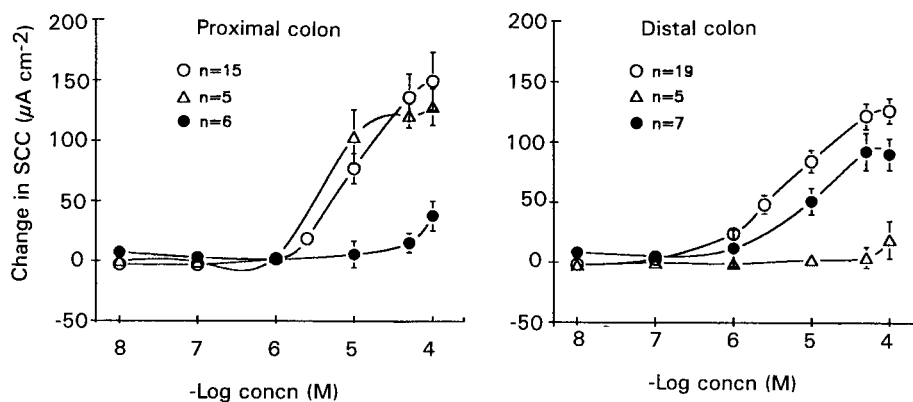

FIG. 3. Effects of 5-HT, 2-Me-5-HT and 5-MT on the SCC generated by stripped sheets of rat proximal and distal colon. The changes in SCC induced by 5-HT (○), 2-Me-5-HT (△) and 5-MT (●) are plotted as a function of log agonist concentration and each point represents the mean ± s.e.m. of the number of observations indicated. In some cases the size of the error bar is less than the size of the symbol.

Table 3. Effects of 5-HT, 2-Me-5-HT and 5-MT on the SCC generated by stripped sheets of proximal and distal colon from the rat.

Experiment	Proximal colon			Distal colon		
	ΔSCC_{max} (μA cm^{-2})	EC50 (μM)	n	ΔSCC_{max} (μA cm^{-2})	EC50 (μM)	n
5-HT	150 ± 24	13 (5·4–33)	15	126 ± 10	4·7 (3·6–6·1)	19
2-Me-5-HT	128 ± 15	66 (1·3–3400)	5	19 ± 16***	—	5
5-MT	38 ± 12*	680*** (19–240000)	6	93 ± 15	11*** (6·6–17)	7

The maximum rises in SCC are expressed as mean ± s.e.m. and the EC50 (geometric mean, 95% confidence limits) of the number of observations indicated. *$P < 0·05$, ***$P < 0·001$ compared with the control value.

($P > 0·05$, Fig. 3, Table 3). 5-MT increased the SCC in both regions of the colon (Fig. 3, Table 3), but was more effective in the distal region where the maximum response was 74 ± 12 (n = 7)% of that to 5-HT compared with only 25 ± 8 (n = 6)% in the proximal region ($P < 0·01$). In both regions of the colon the EC50 values for 5-MT were greater than those obtained with 5-HT, an effect that was more pronounced in the proximal colon (Table 3).

The rise in SCC induced by 5-HT is a reflection of its ability to stimulate electrogenic Cl⁻ secretion (Zimmerman & Binder 1984). Confirmation that this was also the ionic basis of the electrical changes induced by 2-Me-5-HT in the proximal colon and 5-MT in the distal colon was obtained from the observation of reduced responses when frusemide (10^{-3} M in the serosal fluid, 0·05% DMSO v/v in control sheets) was present (10^{-4} M 2-Me-5-HT − control: 89 ± 13

(n = 4) μA cm^{-2}; +frusemide: 41 ± 13 (n = 6) μA cm^{-2}, $P < 0·05$; 10^{-4} M 5-MT − control: 79 ± 12 (n = 6) μA cm^{-2}; + frusemide: 14 ± 6 (n = 5) μA cm^{-2}, $P < 0·01$).

The 5-HT₃ antagonist granisetron (Sanger & Nelson 1989) abolished the response of the proximal colon to 2-Me-5-HT, but failed to influence the ability of 5-HT to increase the SCC in this region (Fig. 4, Table 4), although the higher concentration used exceeded that (10^{-5} M) shown to abolish binding to 5-HT₃-recognition sites in the rat gastrointestinal tract (Champaneria et al 1992). In the distal colon, granisetron did not inhibit the response to 5-HT and at the higher concentration ($1·4 \times 10^{-4}$ M) it reduced the EC50 (Table 4); it also caused a concentration-dependent increase in the maximum response, although this just failed to reach significance (Table 4). Granisetron had little effect on the response of the distal colon to 5-MT (Fig. 4).
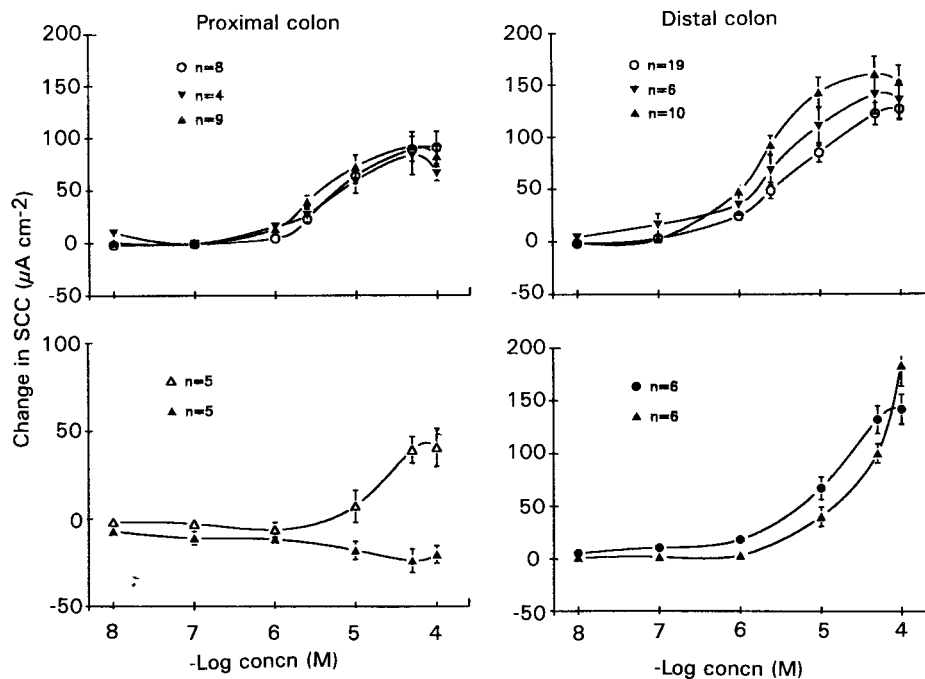

FIG. 4. Effects of granisetron on the response of stripped sheets of rat proximal and distal colon to 5-HT. The effects of granisetron on the proximal colonic response to 2-Me-5-HT and the distal colonic response to 5-MT are also shown. The changes in SCC induced by 5-HT (o), 2-Me-5-HT (△) and 5-MT (●) in the absence and presence of $1·4 \times 10^{-6}$ M (▼) or $1·4 \times 10^{-4}$ M (▲) granisetron are plotted against log agonist concentration and each point represents the mean ± s.e.m. of the number of observations indicated. In some cases the size of the error bar is less than the size of the symbol.

Table 4. Effects of granisetron on the response of stripped sheets of rat proximal and distal colon to 5-HT. Granisetron was added to the serosal solution at the concentrations indicated and 5-HT application began 10 min later.

Experiment	Proximal colon			Distal colon		
	ΔSCC_{max} (μA cm^{-2})	EC50 (μM)	n	ΔSCC_{max} (μA cm^{-2})	EC50 (μM)	n
Control	103 ± 14	6·4 (3·0–14)	8	126 ± 10	4·7 (3·6–6·1)	9
Granisetron ($1·4 \times 10^{-6}$ M)	83 ± 18	5·9 (2·4–15)	4	142 ± 19	3·2 (2·2–4·6)	6
Granisetron ($1·4 \times 10^{-4}$ M)	94 ± 13	3·5 (2·6–4·8)	9	161 ± 17	1·9** (1·6–2·3)	10

The maximum rises in SCC are expressed as mean $\pm$ s.e.m. and the EC50 (geometric mean, 95% confidence limits) of the number of tissues indicated. $**P < 0.01$ compared with the control value.

Discussion

The basal electrical activity of the proximal and distal colon differed. In stripped colonic sheets the basal SCC was higher in the proximal region, while tissue resistance was greater in the distal region. The basal PD was greater in the proximal colon both in-vitro and in-vivo, although the difference between the two regions was not significant, probably because the higher SCC in the proximal colon was offset by a lower tissue resistance. Variations in the pattern of basal ion transport in proximal and distal regions of rat colon have been reported previously (Nobles et al 1991) with the proximal colon secreting Cl$^-$, a process that will contribute to the basal SCC, and the distal colon exhibiting an electroneutral net absorption of Na$^+$ and Cl$^-$. These differences could account for the greater electrical activity observed in the proximal region.

Both proximal and distal regions of the colon responded to 5-HT with an increase in electrical activity, an effect that was observed both in-vivo and in-vitro. The fact that 5-HT induced a rise in SCC in colonic sheets indicates that the response was due to an increase in net electrogenic ion transport and was consistent with its ability to stimulate Cl$^-$ secretion (Zimmerman & Binder 1984).

The involvement of 5-HT$_3$ receptors in the secretory response of proximal and distal colon to 5-HT was examined by investigating the effects of two agonists: 2-Me-5-HT, a selective 5-HT$_3$ agonist (Richardson et al 1985), and 5-MT, an agonist at all 5-HT receptors except 5-HT$_3$ (Fozard 1985; Leff & Martin 1988; Craig et al 1990). The selectivity of their actions was confirmed in-vivo by testing their effects on cardiovascular function. 2-Me-5-HT elicited the Bezold-Jarisch reflex, an effect mediated by 5-HT$_3$ receptors (Kalkman et al 1984), without inducing a pressor or prolonged depressor response. In contrast, 5-MT did not cause an initial bradycardia, but was as effective as 5-HT in inducing the 5-HT$_2$-mediated pressor response and the prolonged depressor response activated by 5-HT$_1$-like receptors.

The effects of these two agonists on transcolonic electrical activity depended on the region investigated and the type of preparation used. 2-Me-5-HT increased the electrical activity of the proximal colon both in-vivo and in-vitro. In both preparations the maximum response was similar to that obtained with 5-HT, but in-vivo the EC50 value was

significantly greater. The increased electrical activity induced by 2-Me-5-HT could be attributed to a stimulation of Cl$^-$ secretion as frusemide, an inhibitor of the Cl$^-$ uptake process at the basolateral membrane (Heintze et al 1983), reduced the response.

5-MT also increased the electrical activity of proximal colon, but it was less effective than 5-HT, with a lower maximum response and a greater EC50 value both in-vivo and in-vitro, differences that were more marked in colonic sheets.

These data suggest that in the proximal colon 5-HT$_3$ receptors make a significant contribution to its secretory response to 5-HT. To test this hypothesis further, the effects of the specific 5-HT$_3$ antagonist granisetron (Sanger & Nelson 1989) were determined. In-vivo it inhibited the Bezold-Jarisch reflex without affecting the pressor and prolonged depressor responses to 5-HT. It also abolished both the cardiovascular and colonic responses to 2-Me-5-HT. Its effects on the 5-HT-induced response of proximal colon were, however, not marked. In-vivo it caused a small decrease in the maximum response without affecting the EC50 value, while in-vitro it was without significant effect. These observations do not, therefore, support the contention that 5-HT$_3$ receptors are the major receptor subtype involved in the secretory response of proximal colon to 5-HT. It is possible that Cl$^-$ secretion can be elicited by more than one 5-HT-receptor subtype and that simply blocking one of these does not lead to loss of the response.

The distal colon exhibited a different pattern of results. In-vivo 2-Me-5-HT increased the PD, although the maximum response was lower and the EC50 value higher than that obtained with 5-HT, while in-vitro it was without effect. In contrast, 5-MT produced similar maximum responses to 5-HT both in-vivo and in-vitro, although the EC50 values were higher. Similar data have been reported for sheets of distal colon (Bunce et al 1991). These findings indicate that 5-HT$_3$ receptors play a less prominent role in the secretory response of the distal colon than they do in proximal colon.

Again the effects of granisetron were more difficult to interpret. This agent abolished the 2-Me-5-HT-induced rise in PD in-vivo and caused a small, but significant reduction in the maximum response to 5-HT without altering the EC50. In-vitro, however, granisetron enhanced the SCC

response to 5-HT, suggesting that 5-HT$_3$ receptors may have an indirect role in the control of secretion. Under normal conditions 5-HT$_3$ receptors might activate an inhibitory pathway designed to limit the magnitude of the secretory response. 5-HT$_3$ receptors are known to be present on sensory nerve endings in the intestinal tract (Fozard 1987) and a recent report has revealed that afferent nerve activity originating from the intestinal mucosa can be stimulated by 5-HT, an effect that is reduced or abolished by granisetron (Blackshaw & Grundy 1993). The present study suggests that one response to this afferent activity might be an inhibition of secretion. Such an inhibitory component in the response to 5-HT has already been indicated in rat small intestine (Beesley & Levin 1991) and colon (Nzegwu & Levin 1990), where an enteric neural adrenergic cholinergic pathway has been proposed. It now seems that this pathway may be activated by 5-HT$_3$ receptors located on sensory nerve endings in the intestinal mucosa.

It is evident from this study that there are differences in the behaviour of in-vivo and in-vitro preparations of rat colon in respect of the actions of 5-HT agonists and antagonists. The maximum response of the proximal colon to 5-MT in-vivo is 73% of that to 5-HT, while in-vitro it only reaches 25%. Similarly, 2-Me-5-HT induces a maximum response in in-vivo preparations of distal colon that is 59% of that to 5-HT, but in-vitro it is without significant effect. Moreover, granisetron causes a small inhibition of the 5-HT response in both regions of the colon in-vivo, but in colonic sheets it has no effect in the proximal colon and has a potentiating effect in the distal colon. Such discrepancies could result from actions of 5-HT agonists and antagonists in-vivo at sites which are absent in the in-vitro preparation. The failure to observe any potentiation of the distal colonic response to 5-HT by granisetron in-vivo could be due to its masking by pro-secretory effects of 5-HT$_3$ receptors exerted at the myenteric plexus, which is removed in stripped colonic sheets, or at sites outside the intestine.

The role of 5-HT$_3$ receptors in the regulation of Cl$^-$ secretion by the distal colon appears to vary in different species. In the guinea-pig this region of the colon behaves more like the proximal colon of the rat, with the 5-HT$_3$ agonist 2-Me-5-HT mimicking the effect of 5-HT in stimulating secretion (Cooke et al 1991).

5-MT is now known to act at 5-HT$_4$ receptors which are considered to be present in the gastrointestinal tract (Craig et al 1990; Eglen et al 1990; Elswood et al 1991; Bockaert et al 1992). Bunce et al (1991) have suggested that 5-HT$_4$ receptors could be responsible for the 5-HT-induced rise in SCC in rat distal colon. This is based on the failure to clearly identify the response as mediated by 5-HT$_1$-like, 5-HT$_2$ or 5-HT$_3$ receptor mechanisms, together with its inhibition by ICS205-930 and the benzamides, metoclopromide and cisapride. However, ICS205-930 antagonizes 5-HT$_3$ receptors as well as 5-HT$_4$ receptors, while the benzamides are reported to act as 5-HT$_4$ agonists (Dumuis et al 1989; Bockaert et al 1992). The ability of 5-MT to elicit a secretory response in rat colon may be taken as evidence for the involvement of 5-HT$_4$ receptors in the control of secretion. It must, however, be remembered that 5-MT also acts at 5-HT$_1$-like and 5-HT$_2$ receptors (Fozard 1985; Hardcastle & Hardcastle 1991), and these could have

contributed to the effects observed in this study. Moreover, recent studies have revealed that 5-HT$_4$ antagonists do not inhibit the colonic response to 5-HT, while the substituted benzamides cisapride and renzapride, which activate 5-HT$_4$ receptors, fail to elicit a secretory response (Franks et al 1993). It therefore seems unlikely that 5-HT$_4$ receptors make a major contribution to the Cl$^-$ secretory response of rat colon to 5-HT.

The present investigation indicates that although 5-HT can induce a secretory response in both proximal and distal colon, the mechanisms responsible differ, with 5-HT$_3$ receptors playing a more prominent pro-secretory role in the proximal region. In the distal colon, 5-HT may activate both pro-secretory and anti-secretory neural pathways. The observed secretory response is, therefore, likely to represent the sum of the effects of several mechanisms activated by 5-HT and these are probably mediated by different 5-HT receptor subtypes. This may explain why antagonism of a single receptor subtype fails to eliminate the secretory effect of 5-HT, although it can abolish the response to more selective agonists.

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