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The Action of 5-Hydroxytryptamine on Normal and Cystic Fibrosis Mouse Colon: Effects on Secretion and Intracellular Calcium

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Abstract

The ability of mouse colon to generate a secretory response to stimulation by 5-hydroxytryptamine (5-HT) was investigated in intact colonic sheets mounted in Ussing chambers. A preparation of intact isolated crypts was used to determine whether 5-HT action was associated with an elevation of cytosolic calcium levels, measured using the calcium-sensitive fluorescent dye, fura-2.

5-HT increased the short-circuit current, an effect that was inhibited by 55% in the absence of chloride and by 83% in the presence of serosal frusemide, consistent with the stimulation of electrogenic chloride secretion. This was confirmed by the observation that colonic tissue from transgenic cystic fibrosis mice ($n = 4$) failed to respond to 5-HT, although wild-type tissues generated an increased short-circuit current of $52{\cdot}4 \pm 11{\cdot}1\ \mu A\,cm^{-2}$ ($n = 9$). The electrical response to 5-HT was calcium-dependent. 5-HT action was unaffected by tetrodotoxin and was not mimicked by the 5-HT_3 agonist 1-phenylbiguanide, indicating that neural mechanisms are not involved. The cyclooxygenase inhibitor indomethacin, however, reduced the 5-HT-induced rise in short-circuit current by 73%, suggesting that prostaglandin production contributes to the response. Stimulation of crypts with acetylcholine elicited an increase in cytosolic calcium levels, but no such response was detected on application of 5-HT (10^{-6} to 10^{-4} M), suggesting that 5-HT does not directly modulate intracellular calcium in colonic crypt cells.

It is concluded that mouse colon responds to 5-HT challenge with a stimulation of electrogenic chloride secretion and that this effect is mediated by indirect mechanisms that might involve immune elements within the colonic wall.

5-Hydroxytryptamine (5-HT) is abundant throughout the intestinal tract. It is found predominantly in the enterochromaffin cells of the mucosa, although it is also present in both neural and immune elements of the subepithelial tissues (McKay & Perdue 1993). 5-HT affects the transport function of the enterocytes that line the intestinal lumen, causing stimulation of electrogenic chloride secretion and inhibition of electroneutral sodium chloride absorption (Hardcastle et al 1981; Zimmerman & Binder 1984), changes that lead to secretion of fluid (Kisloff & Moore 1976; Donowitz et al 1977; Zimmerman & Binder 1984). The mechanisms responsible, however, are still not fully understood; several factors contribute to this uncertainty. The 5-HT receptor population is complex, comprising numerous subtypes (Bradley et al 1986; Hoyer & Schoeffter 1991; Hoyer et al 1994), 5-HT has many possible sites of action within the intestine (Cooke 1994), and there are regional variations in the way the intestine responds to 5-HT challenge (Ayton et al 1995; Hardcastle & Hardcastle 1997a), and species differences (McLean et al 1995).

There is evidence that at least part of the secretory response is mediated indirectly via 5-HT activation of local enteric reflexes (Cooke 1994). However, although 5-HT-induced secretion is inhibited by the neurotoxin, tetrodotoxin, it is not abolished (Cooke & Carey 1985; Baird & Cuthbert 1987; Siriwardena et al 1991; Hardcastle & Hardcastle 1997a, b, 1998), suggesting that a non-neural

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mechanism contributes to the response. Whether this represents a direct action of 5-HT on the transporting cell is still not clear. The secretory actions of 5-HT are considered to be mediated by elevation of cytosolic calcium levels (Bolton & Field 1977; Donowitz et al 1980; Donowitz 1983; Hardcastle et al 1984), but there are few studies in which this has been investigated directly. Fluorescent indicators have been used to demonstrate a 5-HT-induced increase in intracellular calcium levels in isolated chicken enterocytes (Hirose & Chang 1988) and primary cultures of rabbit distal colonic crypt cells (Sahi et al 1996). Similar observations have been reported after use of calcium-sensitive microelectrodes to monitor intracellular calcium levels in an intestinal cell line (Yada et al 1989). In normal mammalian tissues, however, the ability of 5-HT to act directly on the enterocyte has not been established. This study was designed to investigate the secretory response of mouse distal colon to 5-HT challenge and to determine whether 5-HT could modulate cytosolic calcium levels in viable crypts isolated from this tissue.

Materials and Methods

Chemicals

Acetylcholine chloride, frusemide, 5-hydroxytryptamine creatinine sulphate, indomethacin and tetrodotoxin were obtained from Sigma (Poole, UK); 1-phenylbiguanide was from Aldrich (Gillingham, Dorset, UK); dimethylsulphoxide was from BDH Chemicals (Poole, UK); Cell-Tak cell and tissue adhesive was from Becton Dickinson Labware (Bedford, UK), and the acetoxymethyl ester form of the calcium-sensitive fluorescent dye fura-2 was from Molecular Probes (Eugene, OR). All other chemicals were of analytical grade and obtained from commercial suppliers.

Animals

Experiments were performed on male Swiss MF1 mice, 12–13 weeks, 20–30 g, from the Sheffield Field Laboratories. Some experiments were performed on the transgenic cystic fibrosis mouse strain *Cftr*^{tm2Cam} developed by Colledge et al (1995) in which the ΔF508 mutation is introduced into the CFTR gene. These were bred in the Sheffield Field Laboratories and animals used in the study included both wild-type mice and littermates homozygous for the ΔF508 mutation. All mice were allowed free access to food and water, and were killed by cervical dislocation.

Electrophysiology

The potential difference (PD), short-circuit current (SCC) and tissue resistance were measured across sheets of intact distal colon mounted in Ussing chambers with an aperture of 3 mm^2. A small tissue area was chosen to minimize the effects of the regional variations that exist along the length of rodent colon (Nobles et al 1991). Sheets were 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 was determined from the PD change induced by a 10-μA current pulse, taking the fluid resistance into account. 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 30 min after mounting and readings of electrical activity were then taken at 1-min intervals. After 5-min basal readings, 5-HT was added to the serosal solution to give a concentration of 10^{-4} M and readings continued for a further 10 min.

The electrical response to 5-HT was initially tested on one colonic sheet from each animal, taken from the region immediately adjacent to that removed for the preparation of isolated crypts. When the effects of inhibitors were tested, the responses to 5-HT were compared in two sheets taken from adjacent regions of the same colon. The magnitude of the control response to 5-HT in two adjacent sheets did not differ significantly ($P > 0{\cdot}05$). Two pairs of colonic sheets were removed from one animal and for each experimental group the tissues used were from the same region. Each inhibitor was added to the serosal solution of the test sheet as soon as it was set up; the control sheet received an equivalent volume of the vehicle. The inhibitors used were frusemide (10^{-3} M), dissolved in dimethylsulphoxide (0·5% v/v); indomethacin (5×10^{-5} M), dissolved in 1:9 ethanol–0·2% Na_2CO_3 (2% v/v), and tetrodotoxin (10^{-5} M), dissolved in 154 mM NaCl (0·5% v/v). None of the vehicles had a significant effect on the response to 5-HT ($P > 0{\cdot}05$). The effects of lack of chloride were tested by replacing all the chloride in

both mucosal and serosal solutions with gluconate, whereas calcium-free conditions were obtained by omitting calcium from mucosal and serosal solutions and adding 1 mM EGTA.

In experiments on colonic tissue from transgenic cystic fibrosis mice a single colonic sheet was prepared from each animal and the effect of acetylcholine (10^{-3} M) was tested to confirm the genotype. The serosal solution was then washed out and replaced with fresh Krebs bicarbonate saline. After 5 min to enable restabilization, basal readings were taken for 5 min followed by the addition of 5-HT (10^{-4} M) 50 min after the sheet was mounted.

Isolation of colonic crypts

Intact crypts were isolated by an adaptation of the method described by Bjerknes & Cheng (1981) and modified by Doolan & Harvey (1996). A 2–3 cm segment of distal colon, taken from the region immediately adjacent to the rectum, was flushed with 150 mM NaCl (pH 7·4) and tied over a glass rod (2 mm diam.). The glass rod was mounted in a Vibro-Mixer E1 (Chemap AG, Volketswil, Switzerland) and vibrated for 5 s at full speed (vertical amplitude 2–3 mm, 50 Hz) to remove mucosal debris. The colonic segment was then incubated for 30 min at room temperature in 10 mL oxygenated isolation buffer containing 96 mM NaCl, 1·5 mM KCl, 55 mM sorbitol, 44 mM sucrose, 1 mM dithiothreitol, 15 mM EDTA and 13 mM HEPES/Tris at pH 7·4. The colonic segment was then vibrated in the isolation buffer with 4–7 5-s bursts at full speed. Isolated crypts were spun down at 200 g_{max}, and resuspended in 10 mL oxygenated HEPES-buffered Krebs saline (143 mM NaCl, 4·7 mM KCl, 2·5 mM $CaCl_2$, 1·2 mM KH_2PO_4, 1·2 mM $MgSO_4$, 10 mM D-glucose and 20 mM HEPES/Tris at pH 7·4). Crypts were washed by three repeats of this resuspension–centrifugation cycle.

Microfluorimetry

Isolated crypts were transferred to a Cell-Tak-coated cover slip and left to settle for 30 min at room temperature. The cover slip was gently washed with 0·5 mL Krebs HEPES-buffered saline and mounted in a Leiden perfusion chamber. The assembly was mounted in a temperature-controlled PDMI-2 incubation chamber (Medical Systems Corporation, Greenvale, NY) on the stage of an inverted microscope (Olympus IMT-2). A Ratio-Master photometric system (Photon Technology International, Surbiton, UK) was employed. Excitation light from a xenon arc lamp was alternated between 340 and 380 nm wavelengths by use of a monochromator and directed on to the preparation via a 400-nm dichroic long-pass filter and a 40 × PlanApo UV objective. Emitted light was filtered through a 510-nm band-pass filter and directed to a photomultiplier tube detector. Background fluorescence at 340 and 380 nm excitation wavelengths was measured from a selected crypt before loading with fura-2; the values obtained were subtracted from all subsequent measurements. Fura-2 acetoxymethyl ester (5 μM) was added to the chamber and the preparation was left to load for 30 min at room temperature. Crypts were then superfused with oxygenated Krebs HEPES-buffered saline at 37°C. The perfusion rate was 1·5 mL min^{-1}, the chamber volume was 0·5 mL and acquisition speed was 1 point s^{-1}. Traces were smoothed using the Savitzky-Golay algorithm (Savitzky & Golay 1964) in a single pass with a window size of 15 data points. Results are expressed in dimensionless 340 nm/380 nm excitation (340/380) ratios.

Expression of results

Results are expressed as mean values ± s.e.m. of the number of observations indicated. Student's *t*-test, paired or unpaired as appropriate, was used to assess significance.

Results

Electrophysiology

The basal electrical activity generated by colonic sheets from Swiss MF1 mice varied with the region of colon; lower PD and SCC values were observed in the more distal pair of tissues (Table 1). Similar findings have been reported for the rat (Ayton et al 1995). Tissue resistance did not, however, differ in the two regions (Table 1). Serosal application of 5-HT (10^{-4} M) increased both PD and SCC, with little change in tissue resistance; a typical response is shown in Figure 1. The magnitude of the effect was variable, but a positive response was obtained from all tissues, with greater values observed for more distal sheets (Table 1).

The rise in SCC induced by 5-HT was reduced when chloride was omitted from the medium or when frusemide was added to the serosal solution (Table 2), indicating that the electrical response was primarily a result of stimulation of electrogenic chloride secretion. Removal of calcium also reduced the SCC response to 5-HT (Table 2). The effect of 5-HT was not reduced by the neurotoxin

Table 1. Basal electrical activity and the short-circuit current response to 10^{-4} M 5-HT in control sheets taken from the most distal part of the mouse colon (1st pair) and from the adjacent region (2nd pair).

	1st pair (n = 20)	2nd pair (n = 26)	*P*
Basal potential difference (mV)	1·0 ± 0·1	3·3 ± 0·3	< 0·001
Basal short-circuit current (μA cm^{-2})	19·5 ± 1·9	63·2 ± 4·8	< 0·001
Resistance (ohm cm^2)	51·9 ± 2·3	52·2 ± 1·7	> 0·05
Short-circuit current response to 10^{-4} M 5-HT (μA cm^{-2})	52·2 ± 4·8	33·4 ± 5·0	< 0·05

Each value is the mean ± s.e.m. of the number of tissues indicated. An unpaired *t*-test was used to compare the two regions.

Table 2. Short-circuit current response of intact sheets of mouse colon to 5-HT (10^{-4} M) in the absence of mucosal and serosal chloride, the presence of serosal frusemide (10^{-3} M), the absence of mucosal and serosal calcium (+ 1 mM EGTA), the presence of serosal tetrodotoxin (10^{-5} M) or the presence of serosal indomethacin (5×10^{-5} M).

		Short-circuit current response to 5-HT (μA cm^{-2})		
	n	Control	Test	*P*
Chloride-free	6 (2)	33·3 ± 5·2	14·9 ± 4·3	<0·01
Frusemide	8 (1)	55·1 ± 8·4	9·4 ± 2·2	<0·01
Calcium-free	7 (1)	63·2 ± 3·5	27·5 ± 5·3	<0·01
Tetrodotoxin	8 (1)	55·2 ± 9·3	57·1 ± 6·3	>0·05
Indomethacin	7 (2)	42·9 ± 8·2	11·6 ± 2·9	<0·01

Where appropriate, control tissues received an equivalent volume of vehicle. Each value is the mean ± s.e.m. of the number of tissue pairs indicated. A paired *t*-test was used to compare control and test responses. The figures in parentheses indicate whether the 1st (1) or 2nd (2) pair of tissues was used.

tetrodotoxin (Table 2), nor was it mimicked by the 5-HT$_3$ agonist 1-phenylbiguanide (10^{-4} M, change in SCC = 2·7 ± 0·4 μA cm^{-2}, n = 4, $P < 0·001$ compared with the control 5-HT response in the equivalent region of colon), suggesting that neural mechanisms do not contribute to the response. The cyclooxygenase inhibitor indomethacin did inhibit 5-HT action, implicating the involvement of increased prostaglandin production (Table 2).

Basal PD, SCC and tissue resistance values for colonic sheets from wild-type cystic fibrosis mice (3·9 ± 0·5 mV, 69·0 ± 8·3 μA cm^{-2} and 57·1 ± 4·0 ohm cm^2, respectively, n = 9) did not differ significantly ($P > 0·05$ in all cases) from those observed in the equivalent region of colon (2nd pair of sheets—Table 1) from Swiss MF1 mice. In tissues from ΔF508 cystic fibrosis animals basal PD and SCC were much lower than those in wild-type colon (0·2 ± 0·2 mV and 10·8 ± 9·1 μA cm^{-2}, n = 4, $P < 0·001$ in both cases), although tissue resistance was similar (41·1 ± 6·7 ohm cm^2, $P > 0·05$). Acetylcholine (10^{-3} M) caused an increase in the SCC in each wild-type colon, with a mean change of 17·4 ± 4·8 μA cm^{-2} (n = 9), but it did not increase the SCC of ΔF508 cystic fibrosis tissues (−1·4 ± 0·8 μA cm^{-2}, n = 4, $P < 0·05$). These findings mirror the behaviour of rectal mucosa from cystic fibrosis patients (Hardcastle et al 1991). Similarly 5-HT (10^{-4} M) increased the SCC of wild-type tissues (mean increase = 52·4 ± 11·1 μA cm^{-2}, n = 9), but had no effect in cystic fibrosis tissues (0·0 ± 0·0 μA cm^{-2}, n = 4, $P < 0·05$, Figure 2).

Microfluorimetry

Cells of the isolated crypts were viable as indicated by their ability to exclude trypan blue and propidium iodide. Their viability was confirmed by a transient rise in the 340/380 ratio in fura-2-loaded crypts, an indication of increased cytosolic calcium levels, in response to 10^{-4} M acetylcholine (Figure 3). The peak 340/380 value occurred 90 ± 12 s after the start of acetylcholine superfusion and the ratio increased significantly ($P < 0·001$), with a maximum change of 0·22 ± 0·03 (n = 5). In contrast, 5-HT, at concentrations ranging from 10^{-6} to 10^{-4} M, failed to influence cytosolic calcium levels. This cannot be attributed to a decrease in crypt-cell viability during prolonged superfusion, because a second acetylcholine pulse, delivered after completion of the 5-HT applications, elicited a calcium response (although reduced in time and amplitude) in 3 of 5 preparations (Figure 3). Colonic sheets prepared from the region immediately adjacent to that used to isolate crypts each generated a

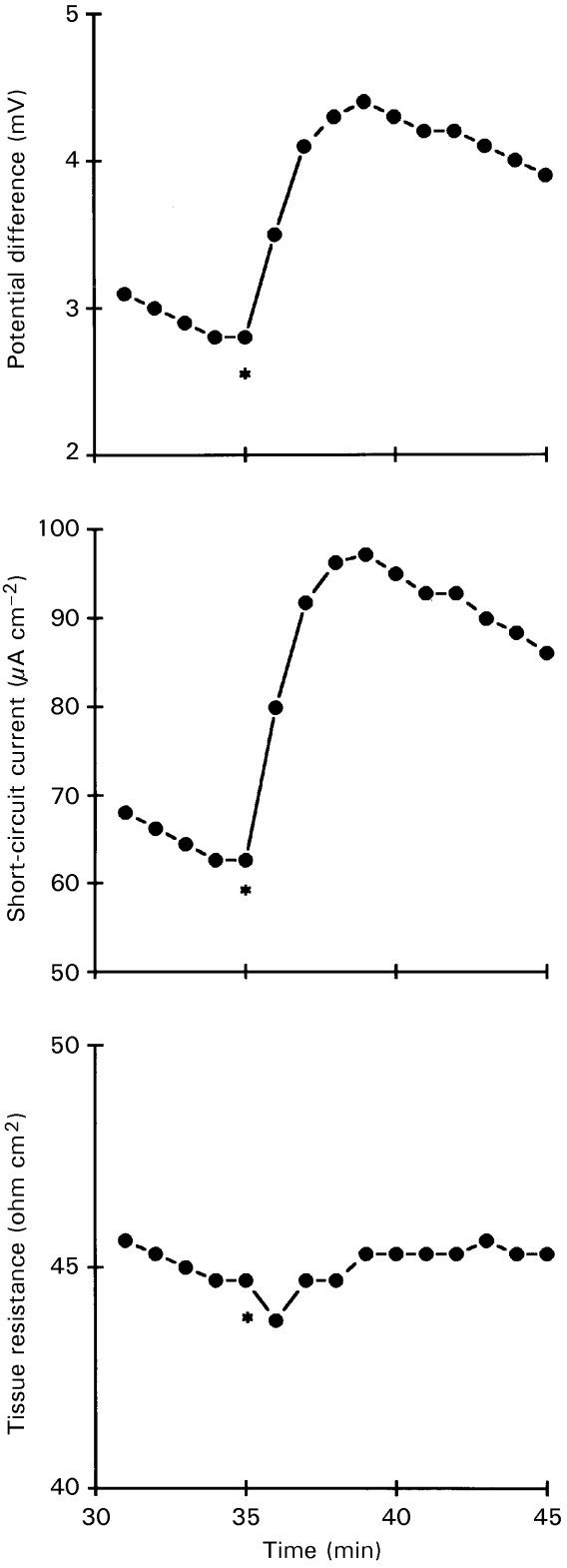

Figure 1. Typical response of intact sheets of mouse colon to 5-HT (10^{-4} M) challenge. 5-HT was added to the serosal solution at 35 min (*) and the changes in the potential difference, short-circuit current and tissue resistance are plotted as a function of time.

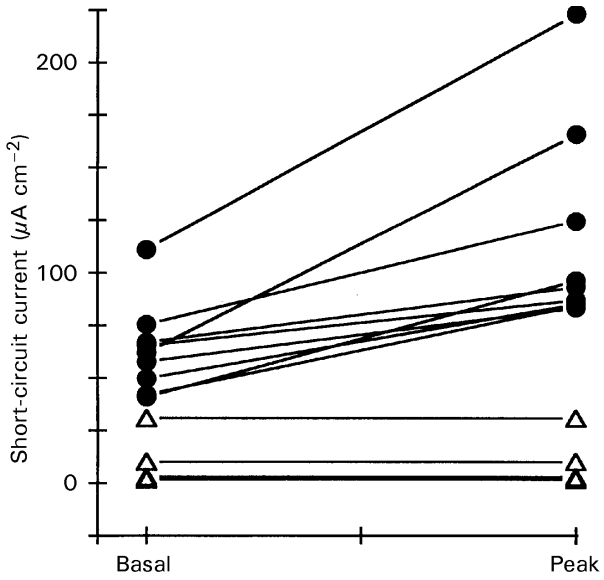

Figure 2. Changes in short-circuit current induced by serosal application of 5-HT (10^{-4} M) to intact sheets of colon from cystic fibrosis (*Cftr*^{tm2Cam}) mice. Short-circuit current values immediately before adding 5-HT and at the peak of the response are shown for nine sheets from wild-type mice (●) and four sheets from mice homozygous for the ΔF508 mutation (Δ).

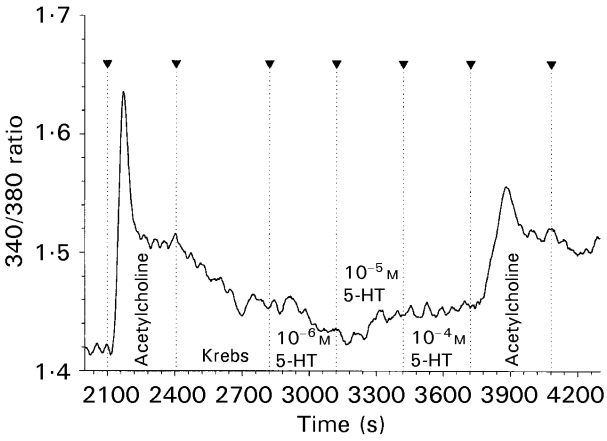

Figure 3. Typical changes in cytosolic calcium levels in response to acetylcholine (10^{-4} M) and 5-HT (10^{-6}–10^{-4} M) in crypts isolated from mouse colon. Crypt cells were loaded with fura-2 and cytosolic calcium is expressed as dimensionless 340/380 ratios.

significant increase in SCC on exposure to 10^{-4} M 5-HT ($26{\cdot}0 \pm 7{\cdot}5\ \mu A\ cm^{-2}$, n = 5, $P < 0{\cdot}05$).

Discussion

The electrophysiological studies confirm that mouse colon, like that of rat (Zimmerman & Binder 1984; Bunce et al 1991; Siriwardena et al 1991; Ayton et al 1995) and guinea-pig (Cooke et al 1991) responds to 5-HT challenge with an increased SCC that is consistent with the stimulation

of electrogenic chloride secretion. This was confirmed by the finding that the rise in SCC induced by 5-HT was lower in the absence of chloride or when the basolateral Na−K−2Cl transporter, an integral part of the chloride secretory process (Heintze et al 1983), was inhibited by frusemide. It is now well established that intestinal tissue from transgenic cystic fibrosis mice has the secretory defect that occurs in the intestine of patients (Grubb & Gabriel 1997) and this was confirmed in the current study—acetylcholine increased the SCC in colonic sheets from wild-type but not from ΔF508 cystic fibrosis mice. That 5-HT failed to increase the SCC in cystic fibrosis colon, although wild-type tissues generated a normal response, is further evidence that in normal tissues 5-HT elicits stimulation of chloride secretion.

The magnitude of the response to 5-HT was variable, but this is not entirely unexpected, because of the multiplicity of mechanisms involved. There is evidence for both prosecretory and antisecretory components in the actions of 5-HT in intact intestinal sheets (Hardcastle & Hardcastle 1997b) and it is likely that the balance between the various processes that contribute to the overall response might vary in different preparations. Nevertheless, each sheet exhibited a positive SCC response when 5-HT was added.

The question of whether 5-HT has direct action on the enterocyte has yet to be fully resolved, despite several lines of inquiry. The first study to investigate the possibility that 5-HT receptors were present on intestinal epithelial cells used a variety of 5-HT ligands, but failed to demonstrate any specific binding sites on membranes prepared from enterocytes isolated from rat ileum (Gaginella et al 1983). A later study using a more selective antagonist identified 5-HT_2 receptors linked to the production of inositol trisphosphate in isolated crypt cells from guinea-pig small intestine (Siriwardena et al 1993). Other studies have reported functional effects of 5-HT on enterocytes. In crypt and villous cells from rabbit ileum 5-HT has been shown to influence Na^+-H^+ and $Cl^-{-}HCO_3^-$ exchange mechanisms (Sundaram et al 1991), and a patch-clamp study in isolated crypts from rat colon has demonstrated changes in whole-cell potassium and chloride currents mediated by 5-HT_2 receptors and involving a G-protein-coupled pathway (Imada-Shirakata et al 1997). In contrast, 5-HT has a negligible effect on the SCC generated by T84 cells when they are cultured alone, but elicits a significant electrical response when they are co-cultured with fibroblasts. This effect was inhibited by indomethacin, suggesting the involvement of prostaglandins released by immune cells (Berschneider & Powell 1992). This is consistent with data from an earlier study, in which 5-HT was found to elicit both fluid secretion and an increased PGE_2 output into the lumen of rat jejunum in-vivo (Beubler et al 1986). The results of the current study suggest that prostaglandins are also involved in the secretory response of mouse colon to 5-HT as indomethacin inhibited the SCC response. In contrast, the 5-HT response in mouse colon does not seem to involve activation of neural pathways, because tetrodotoxin did not reduce the 5-HT-induced rise in SCC, and the 5-HT_3 agonist 1-phenylbiguanide, which acts solely via neural mechanisms (Hardcastle & Hardcastle 1997b), had little effect on the SCC. Similar findings have been reported in rat distal colon (Ayton et al 1995). Other studies in rat (Siriwardena et al 1991) and guinea-pig (Cooke et al 1991) colon have, however, detected a tetrodotoxin-sensitive component in the electrical response to 5-HT, but it is possible that the region of colon tested was located more proximally than that used in the current investigation. There is therefore, evidence for both direct and indirect actions of 5-HT on the transporting cells of the colon.

Intestinal secretion can be stimulated by both cyclic nucleotides and calcium. It has been shown that 5-HT has no effect on the production of cyclic AMP by isolated enterocytes (Hardcastle et al 1981) and it is generally considered that 5-HT acts by elevation of cytosolic calcium levels (Bolton & Field 1977; Donowitz et al 1980; Donowitz 1983; Hardcastle et al 1984). This conclusion is based on experiments performed in intact tissues under calcium-free conditions or with calcium-channel blockers; similar experiments in this study have demonstrated that the secretory response of intact mouse colon to 5-HT is also calcium-dependent. The effects of these conditions however, are not necessarily exerted at the level of the enterocyte. Direct measurement of intracellular calcium levels has been performed only on cultured cells (Yada et al 1989; Sahi et al 1996) or on cells isolated from chicken small intestine (Hirose & Chang 1988). In the current study microfluorimetry was used to monitor cytosolic calcium in an isolated crypt preparation from mammalian colon. The data clearly demonstrate the absence of a calcium signal in response to 5-HT challenge, because cytosolic calcium levels did not change in the presence of a range of 5-HT concentrations. The ability of the preparation to generate a calcium signal was confirmed by testing the effects of acetylcholine. Cholinergic agonists induce intestinal secretion via muscarinic receptors that are present on the enterocytes (Lundgren 1988) and they have been shown

to increase intracellular calcium in crypts isolated both from mouse colon and from human colon (Pennec et al 1996). In the current study acetylcholine generated a clear calcium signal so the failure of 5-HT to produce a similar response cannot be attributed to a lack of responsiveness of the preparation. It seems, therefore, that in mouse colon 5-HT does not elicit its secretory response by directly increasing calcium levels within the enterocyte.

The results of this study indicate that although 5-HT elicits a secretory response from intact sheets of mouse colon, it is unable to generate a cytosolic calcium signal in isolated crypts. This suggests that 5-HT is not having a direct action on the crypt cells of mouse colon and that the secretory response is mediated by indirect mechanisms that probably involve prostaglandin production by immune cells within the intestinal wall.

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References

Ayton, B., Hardcastle, J., Hardcastle, P. T., Carstairs, J. W. M. (1995) Comparison of the secretory actions of 5-hydroxytryptamine in the proximal and distal colon of the rat. J. Pharm. Pharmacol. 47: 34–41

Baird, A. W., Cuthbert, A. W. (1987) Neuronal involvement in type I hypersensitivity reactions in gut epithelia. Br. J. Pharmacol. 92: 647–655

Berschneider, H. M., Powell, D. W. (1992) Fibroblasts modulate intestinal secretory response to inflammatory mediators. J. Clin. Invest. 89: 484–489

Beubler, E., Bukhave, K., Rask-Madsen, J. (1986) Significance of calcium for the prostaglandin E_2-mediated secretory response to 5-hydroxytryptamine in the small intestine of the rat in vivo. Gastroenterology 90: 1972–1977

Bjerknes, M., Cheng, H. (1981) Methods for the isolation of intact epithelium from the mouse intestine. Anat. Rec. 199: 565–574

Bolton, J. E., Field, M. (1977) Ca ionophore-stimulated ion secretion in rabbit ileal mucosa: relation to cyclic 3′,5′-AMP and carbamylcholine. J. Membrane Biol. 35: 159–173

Bradley, P. B., Engel, G., Fenuik, W., Fozard, J. R., Humphrey, P. P. A., Middlemiss, D. N., Mylecharane, E. J., Richardson, B. P., Saxena, P. R. (1986) Proposals for the classification and nomenclature of functional receptors for 5-hydroxytryptamine. Neuropharmacology 25: 563–576

Bunce, K. T., Elswood, C. J., Ball, M. T. (1991) Investigation of the 5-hydroxytryptamine receptor mechanism mediating the short-circuit current response in rat colon. Br. J. Pharmacol. 102: 811–816

Colledge, W. H., Abella, B. S., Southern, K. W., Ratcliff, R., Jiang, C., Cheng, S. H., MacVinish, L. J., Anderson, J. R., Cuthbert, A. W., Evans, M. J. (1995) Generation and characterization of a ΔF508 cystic fibrosis mouse model. Nature Gen. 10: 445–452

Cooke, H. J. (1994) Neuroimmune signaling in regulation of intestinal ion transport. Am. J. Physiol. 266: G167–G178

Cooke, H. J., Carey, H. V. (1985) Pharmacological analysis of 5-hydroxytryptamine actions on guinea-pig ileal mucosa. Eur. J. Pharmacol. 111: 320–337

Cooke, H. J., Wang, Y.-Z., Frieling, T., Wood, J. D. (1991) Neural 5-hydroxytryptamine receptors regulate chloride secretion in guinea pig distal colon. Am. J. Physiol. 261: G833–G840

Donowitz, M. (1983) Ca^{2+} in the control of active intestinal Na and Cl transport: involvement in neurohumoral action. Am. J. Physiol. 245: G165–G177

Donowitz, M., Charney, A. N., Heffernan, J. M. (1977) Effect of serotonin treatment on intestinal transport in the rabbit. Am. J. Physiol. 232: E85–E94

Donowitz, M., Asarkof, N., Pike, G. (1980) Calcium dependence of serotonin-induced changes in rabbit ileal electrolyte transport. J. Clin. Invest. 66: 341–352

Doolan, C. M., Harvey, B. J. (1996) Modulation of cytosolic protein kinase C and calcium ion activity by steroid hormones in rat distal colon. J. Biol. Chem. 271: 8763–8767

Gaginella, T. S., Rimele, T. J., Weitecha, M. (1983) Studies on rat intestinal epithelial cell receptors for serotonin and opiates. J. Physiol. 335: 101–111

Grubb, B. R., Gabriel, S. E. (1997) Intestinal physiology and pathology in gene-targeted mouse models of cystic fibrosis. Am. J. Physiol. 273: G258–G266

Hardcastle, J., Hardcastle, P. T. (1997a) Comparison of the intestinal secretory response to 5-hydroxytryptamine in the rat jejunum and ileum in-vitro. J. Pharm. Pharmacol. 49: 1126–1131

Hardcastle, J., Hardcastle, P. T. (1997b) Several receptor subtypes contribute to 5-hydroxytryptamine-induced secretion by rat ileum in-vitro. J. Pharm. Pharmacol. 49: 1114–1120

Hardcastle, J., Hardcastle, P. T. (1998) 5-Hydroxytryptamine-induced secretion by rat jejunum in-vitro involves several 5-hydroxytryptamine receptor subtypes. J. Pharm. Pharmacol. 50: 539–547

Hardcastle, J., Hardcastle, P. T., Redfern, J. S. (1981) Action of 5-hydroxytryptamine on intestinal ion transport in the rat. J. Physiol. 320: 41–55

Hardcastle, J., Hardcastle, P. T., Noble, J. M. (1984) The involvement of calcium in the intestinal response to secretagogues in the rat. J. Physiol. 355: 465–478

Hardcastle, J., Hardcastle, P. T., Taylor, C. J., Goldhill, J. (1991) Failure of cholinergic stimulation to induce a secretory response from the rectal mucosa in cystic fibrosis. Gut 32: 1035–1039

Heintze, K., Stewart, C. P., Frizzell, R. A. (1983) Sodium-dependent chloride secretion across rabbit descending colon. Am. J. Physiol. 244: G357–G365

Hirose, R., Chang, E. B. (1988) Effects of serotonin on Na^+-H^+ exchange and intracellular calcium in isolated chicken enterocytes. Am. J. Physiol. 254: G891–G897

Hoyer, D., Schoeffter, P. (1991) 5-HT receptors: subtypes and second messengers. J. Receptor Res. 11: 197–214

Hoyer, D., Clarke, D. E., Fozard, J. R., Hartig, P. R., Martin, G. R., Mylecharane, E. J., Saxena, P. R., Humphrey, P. P. A. (1994) International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (serotonin). Pharmacol. Rev. 46: 157–203

Imada-Shirakata, Y., Kotera, T., Ueda, S., Okuma, M. (1997) Serotonin activates electrolyte transport via $5\text{-}HT_{2A}$ receptor in rat colonic crypt cells. Biochem. Biophys. Res. Commum. 230: 437–441

Kisloff, B., Moore, E. W. (1976) Effect of serotonin on water and electrolyte transport in the in-vivo rabbit small intestine. Gastroenterology 71: 1033–1038

Lundgren, O. (1988) Nervous control of intestinal transport. Baillière's Clin. Gastroenterol. 2: 85–106

McKay, D. M., Perdue, M. H. (1993) Intestinal epithelial function: the case for immunophysiological regulation. Cells and mediators. Dig. Dis. Sci. 38: 1377–1387

McLean, P. G., Coupar, I. M., Molenaar, P. (1995) A comparative study of functional 5-HT_4 receptors in human colon, rat oesophagus and rat ileum. Br. J. Pharmacol. 115: 47–56

Nobles, M., Diener, M., Mestres, P., Rummel, W. (1991) Segmental heterogeneity of the rat colon in the response to activators of secretion on the cAMP-, the cGMP- and the Ca^{2+}-pathway. Acta Physiol. Scand. 142: 375–386

Pennec, J. P., Mignen, O., Harvey, B., Thomas, S. (1996) Effets du carbachol et de l'histamine sur le potentiel de membrane et le calcium intracellulaire des cellules des cryptes de colon distal humain et de souris. Arch. Physiol. Biochem. 104: D132

Sahi, J., Wiggins, M. P., Gibori, G. B., Layden, T. J., Rao, M. C. (1996) Calcium regulated chloride permeabilities in primary cultures of rabbit colonocytes. J. Cell. Physiol. 168: 276–283

Savitzky, A., Golay, M. J. E. (1964) Smoothing and differentiation of data by simplified least square procedures. Anal. Chem. 36: 1627–1639

Siriwardena, A. K., Booker, C., Pratt, J., Kellum, J. M. (1991) Pathways of serotonin-induced electrolyte transport in rat distal colon. Surgery 110: 411–418

Siriwardena, A. K., Smith, E. H., Borum, E. H., Kellum, J. M. (1993) Identification of a 5-hydroxytryptamine (5-HT_2) receptor on guinea pig small intestinal crypt cells. Am. J. Physiol. 265: G339–G346

Sundaram, U., Knickelbein, R. G., Dobbins, J. W. (1991) Mechanism of intestinal secretion. Effect of serotonin on rabbit ileal crypt and villus cells. J. Clin. Invest. 87: 743–746

Yada, T., Oiki, S., Ueda, S., Okada, Y. (1989) Intestinal secretagogues increase cytosolic free Ca^{2+} concentration and K^{+} conductance in a human intestinal epithelial cell line. J. Membrane Biol. 112: 159–167

Zimmerman, T. W., Binder, H. J. (1984) Serotonin-induced alteration of colonic electrolyte transport in the rat. Gastroenterology 86: 310–317