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# Comparison of the Intestinal Secretory Response to 5-Hydroxytryptamine in the Rat Jejunum and Ileum In-vitro

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## Abstract

A secretory response to 5-hydroxytryptamine (5-HT) is observed throughout the intestinal tract; this investigation has compared the nature of this response in the jejunum and ileum of the rat in-vitro.

Different basal electrical activity was observed for jejunal and ileal sheets of rat small intestine. In both intact and stripped preparations the basal short-circuit current (SCC) was greater and the tissue resistance lower in the jejunum than in the ileum. 5-HT caused concentration-dependent increases in SCC in intact and stripped preparations of both regions. EC50 values were similar in the jejunum and ileum, stripped sheets from both regions showing greater sensitivity. In the ileum the maximum increase in SCC induced by 5-HT was similar in intact and stripped sheets, but in the jejunum the response was greater in intact preparations. The jejunal response to 5-HT was reduced in the absence of bicarbonate but unaffected by lack of chloride, whereas the ileal response was inhibited by removal of chloride but unaltered in bicarbonate-free conditions. In intact sheets the tetrodotoxin-sensitive neural component was greater in the jejunum. In stripped sheets a neural component could still be detected in the ileum, but not in the jejunum.

There are, therefore, fundamental differences in the way in which the jejunum and ileum respond to 5-HT stimulation—the jejunal response is primarily a result of stimulation of bicarbonate secretion whereas chloride secretion predominates in the ileum. The myenteric plexus appears to play a more prominent role in the jejunum; in the ileum other neural elements also contribute to the response.

Intestinal secretion induced by 5-hydroxytryptamine (5-HT) stimulation is complex, with both neural and non-neural components contributing to the response (McKay & Perdue 1993; Cooke 1994; Franks et al 1996). There is, moreover, a considerable number of 5-HT receptor subtypes (Bradley et al 1986; Hoyer & Schoeffter 1991; Hoyer et al 1994) and evidence is emerging that several of these might be involved in the intestinal response to 5-HT challenge (Scott et al 1992; Ayton et al 1995; Hardcastle & Hardcastle 1995, 1996a). Thus 5-$HT_2$ (Beubler & Horina 1990; Beubler et al 1990, 1993; Siriwardena & Kellum 1993), 5-$HT_3$ (Baird & Cuthbert 1987; Beubler & Horina 1990; Cooke et al 1991; Beubler et al 1993) and 5-$HT_4$ (Borman & Burleigh 1993; Burleigh & Borman 1993; Budhoo & Kellum 1994) receptors have all been implicated in 5-HT action in the gut, although the location of the various subtypes within the intestine has yet to be clarified. There is general agreement that 5-$HT_3$ receptors in the intestinal tract are located on sensory neurones (Fozard 1987) and activate a cholinergic mechanism to stimulate secretion (Hendriks et al 1989; Cooke et al 1991). However, 5-$HT_3$ antagonists do not abolish the response to 5-HT, although they do eliminate 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 inducing a secretory response (Hardcastle & Hardcastle 1991, 1995). There is some evidence that 5-HT has a direct action on the transporting cells. In guinea-pig small intestine 5-$HT_2$ receptors, linked to the production of inositol tris phosphate ($IP_3$), have been located on the crypt cells (Siriwardena et al 1993) and in chicken small intestine a 5-HT-induced rise in cytoplasmic calcium levels, measured fluorimetrically, has been reported in isolated enterocytes (Hirose & Chang 1988). It therefore appears that in its stimulation of intestinal secretion 5-HT might have both direct and indirect actions. The situation is further complicated by regional differences in response to 5-HT. In pig small intestine the magnitude of the response to 5-HT varies along the length of the gut (Grondahl et al 1996), whereas in rat proximal and distal colon the receptors responsible for the 5-HT-induced rise in transintestinal electrical activity both in-vivo and in-vitro are different, with 5-$HT_3$ receptors making a greater contribution in the proximal region (Ayton et al 1995). There is also an indication that there might be differences in the ionic basis of the response in the jejunum and ileum (Donowitz et al 1980; Hardcastle et al 1981; Urquhart et al 1988). The current study was therefore designed to investigate further regional differences in 5-HT-induced secretion in rat small intestine by comparing the responses of the jejunum and ileum to 5-HT challenge. A preliminary report of this work has been published (Hardcastle & Hardcastle 1996b).

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## Materials and Methods

### *Chemicals*

5-Hydroxytryptamine creatinine sulphate, furosemide and tetrodotoxin were obtained from Sigma (Poole, Dorset, UK) and dimethylsulphoxide from BDH (Poole, Dorset, UK). All drugs were dissolved in 154 mM NaCl except for furosemide for which the vehicle was dimethylsulphoxide.

*Animals*

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

*Measurement of transintestinal electrical activity across ileal sheets*

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

The tissues were left to stabilize for 10 min after mounting and then electrical activity was measured at 1-min intervals. After 5 min basal readings 5-HT agonists were added to the serosal solution. Cumulative concentration–response curves were constructed by applying the next concentration of agonist at the peak of the response to the previous application, as described by Bunce et al (1991). When the effects of furosemide or tetrodotoxin were investigated the drug was added to the serosal solution of the test sheet as soon as the sheets were set up, with control sheets receiving an equivalent volume of vehicle. To test tissue viability and possible non-specific actions of the test conditions, glucose (10 mM) was added to the mucosal solution of both sheets 10 min after the final addition of agonist. In experiments performed to investigate the ionic basis of the 5-HT response the composition of the bathing medium was changed. The effects of lack of chloride were tested by replacing chloride in the serosal solution with gluconate; under bicarbonate-free conditions a Krebs phosphate buffer, oxygenated with 100% $O_2$, was used on both sides of the tissue. When present, furosemide was added to the serosal solution of test sheets at a concentration of 1 mM while control sheets received an equivalent volume of vehicle (dimethylsulphoxide, 0·5% v/v). Preliminary experiments indicated that the vehicle alone did not inhibit the response to 5-HT.

*Expression of results*

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

## Results

*Basal electrical activity in intact and stripped jejunum and ileum*

In intact and stripped preparations the basal SCC was greater and the tissue resistance lower in the jejunum than in the ileum (Table 1). The intact jejunum generated a higher PD than the intact ileum, but in stripped preparations PD values were similar in the two regions (Table 1). Removal of the muscle layers and myenteric plexus had different effects on basal electrical activity in the two regions. In the jejunum all three indices were significantly reduced, whereas in the ileum PD and SCC values rose but the tissue resistance remained unchanged (Table 1).

*Response to 5-HT*

5-HT induced concentration-dependent increases in the SCC of both intact and stripped preparations of jejunum and ileum (Fig. 1). In intact preparations jejunal and ileal responses were similar, with maximum increases in SCC of 61·2 ± 8·4 μA $cm^{-2}$ (n = 26) in the jejunum and 49·8 ± 5·4 μA $cm^{-2}$ (n = 30) in the ileum ($P > 0.05$). Corresponding EC50 values were 12·8 (9·6–17·1) μM and 12·5 (9·7–16·1) μM. The stripped preparations were more sensitive to the action of 5-HT, with EC50 values being lower for both the jejunum (4·1 (3·1–5·4) μM; n = 27; $P < 0.001$) and the ileum (3·2 (2·6–3·9) μM; n = 30; $P < 0.001$). Again values did not differ significantly in the two regions ($P > 0.05$). In the jejunum the maximum

Table 1. Basal electrical activity of intact and stripped sheets of rat jejunum and ileum.

| | | Number of observations | Potential difference (mV) | SCC (-μA $cm^{-2}$) | Resistance (ohm $cm^2$) |
|---|---|---|---|---|---|
| Intact | Jejunum | 58 | 2·4 ± 0·1 | 62·7 ± 3·2 | 40·5 ± 1·4 |
| | Ileum | 56 | 1·0 ± 0·1 | 15·6 ± 2·1 | 61·2 ± 2·3 |
| | | | $P < 0.001$ | $P < 0.01$ | $P < 0.001$ |
| Stripped | Jejunum | 57 | 1·5 ± 0·1‡ | 49·6 ± 2·8† | 29·6 ± 0·9‡ |
| | Ileum | 62 | 1·5 ± 0·1† | 25·5 ± 2·4† | 57·9 ± 1·8 |
| | | | $P > 0.05$ | $P < 0.001$ | $P < 0.001$ |

Each value was measured 15 min after the sheets were mounted and represents the mean ± s.e.m. of the number of observations indicated. All potential difference and SCC values are serosa positive. An unpaired *t*-test was used to assess the significance of any differences observed. *P*-values in the table relate to comparison of values from the jejunum and ileum. † $P < 0.01$, ‡ $P < 0.001$, significant difference between results from intact and stripped preparations.

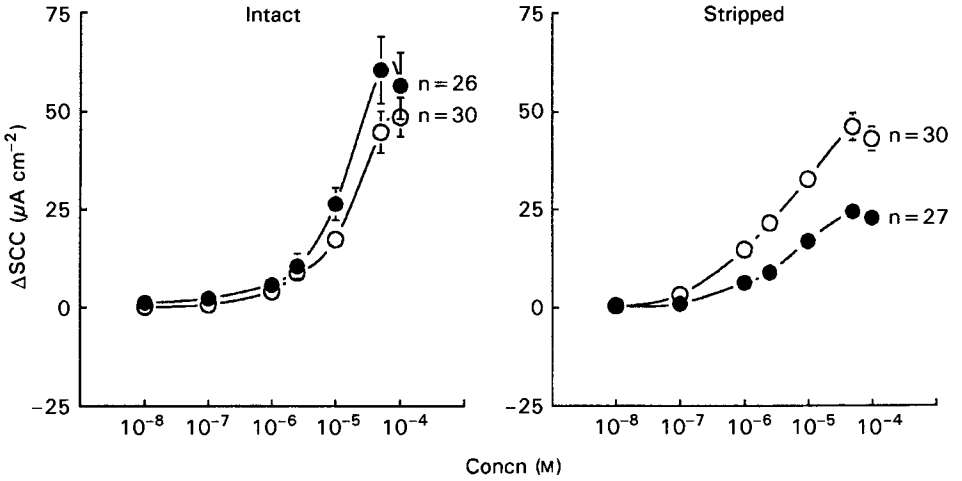


FIG. 1. Concentration-dependence of action of 5-HT on intact and stripped sheets of rat jejunum (●) and ileum (○). The increases in SCC (ΔSCC) induced by cumulative additions of 5-HT are plotted as a function of log agonist concentration and each point represents the mean ± s.e.m. of the number of observations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

increase in SCC was reduced in the stripped preparation to 25·6 ± 2·6 μA cm$^{-2}$ ($P < 0.001$), although in the stripped ileum the maximum response (46·5 ± 3·5 μA cm$^{-2}$) was not reduced significantly from the value obtained for intact sheets ($P > 0.05$). In the stripped preparation, therefore, the maximum SCC change was greater in the ileum than in the jejunum ($P < 0.001$).

*Ionic basis of the response to 5-HT*

In both intact and stripped sheets of jejunum removal of chloride from the serosal solution or the presence of furosemide failed to inhibit the increase in SCC induced by 5-HT, although in the ileum these conditions significantly reduced the 5-HT response (Fig. 2). In contrast, bicarbonate-free conditions caused a marked decrease in the jejunal response to 5-HT, without affecting the ileal response (Fig. 2). The response to 10 mM luminal glucose (intact jejunum 125·0 ± 14·6 μA cm$^{-2}$, n = 28; stripped jejunum 127·4 ± 8·2 μA cm$^{-2}$, n = 24; intact ileum 100·6 ± 11·8 μA cm$^{-2}$, n = 22; stripped ileum 103·4 ± 6·1 μA cm$^{-2}$, n = 24) was unaffected by removal of serosal chloride or the presence of furosemide in either preparation of jejunum or ileum ($P > 0.05$ in all cases). The absence of bicarbonate reduced the glucose response in both intact (control 107·6 ± 12·5 μA cm$^{-2}$; bicarbonate-free 30·9 ± 6·5 μA cm$^{-2}$, n = 8; $P < 0.01$) and stripped (control 155·2 ± 15·4 μA cm$^{-2}$; bicarbonate-free 88·6 ± 7·5 μA cm$^{-2}$, n = 8; $P < 0.01$) preparations of jejunum, although in the ileum the glucose response was unaffected by these conditions ($P > 0.05$ for both). However, the extent of inhibition of the response to glucose in intact jejunum was significantly less than that to 5-HT (glucose 70·9 ± 6·1%; 5-HT 83·3 ± 5·0%, n = 8; $P < 0.05$), although in the stripped preparation the difference was not significant ($P > 0.05$).

*Effect of tetrodotoxin on the response to 5-HT*

In the jejunum tetrodotoxin reduced the basal SCC in intact sheets by 47 ± 5% (n = 12; $P < 0.001$), but was without effect in stripped preparations ($P > 0.05$). In the ileum tetrodotoxin had no significant effect on the basal SCC in either preparation ($P > 0.05$ for both). However, in both intact and stripped sheets the basal SCC in the presence of the neurotoxin was lower in the ileum than in the jejunum (intact jejunum 31·0 ± 3·3 μA cm$^{-2}$, n = 12; intact ileum 11·2 ± 3·1 μA cm$^{-2}$, n = 12; $P < 0.001$; stripped jejunum 46·5 ± 4·8 μA cm$^{-2}$, n = 7; stripped ileum 18·7 ± 2·5 μA cm$^{-2}$, n = 8; $P < 0.001$). Tetrodotoxin did not alter tissue resistance in any preparation ($P > 0.05$ in all cases). Tetrodotoxin caused a significant inhibition of the response to 5-HT in intact preparations of both jejunum and ileum (Fig. 3). The extent of inhibition was, however, more marked in the jejunum (jejunum 86·3 ± 1·9%, n = 4; ileum 57·4 ± 2·7%, n = 4; $P < 0.001$). Stripping the intestine reduced the extent of inhibition in the ileum (to 25·0 ± 8·2%, n = 8, $P < 0.05$ compared with intact sheets) and abolished it in the jejunum. The residual response to 5-HT in the presence of tetrodotoxin was increased by stripping in both the jejunum (intact 11·3 ± 0·5 μA cm$^{-2}$, n = 4; stripped 28·8 ± 5·6 μA cm$^{-2}$, n = 6; $P < 0.05$) and the ileum (intact 25·9 ± 2·3 μA cm$^{-2}$, n = 4; stripped 44·2 ± 1·3 μA cm$^{-2}$, n = 8; $P < 0.001$), although in the absence of tetrodotoxin stripping had no effect in the ileum (intact 61·3 ± 5·8 μA cm$^{-2}$, n = 4; stripped 63·2 ± 6·2 μA cm$^{-2}$, n = 8; $P > 0.05$) and reduced the response in the jejunum (intact 87·6 ± 15·1 μA cm$^{-2}$, n = 4; stripped 39·5 ± 6·4 μA cm$^{-2}$, n = 6; $P < 0.01$).

## Discussion

This study has demonstrated that for both basal and 5-HT-stimulated transintestinal electrical activity there are clear differences between values measured in the jejunum and ileum of the rat. Under basal conditions the SCC was lower and the tissue resistance higher in the ileum in both intact and stripped preparations. The increased tissue resistance in more distal regions of the small intestine is a reflection of the reduced permeability of the paracellular pathway, the main determinant of transintestinal conductivity (Schultz et al 1974). Neural mechanisms contributed to the greater basal SCC in intact jejunal sheets, because tetrodotoxin caused a reduction of 47%. Most of this neural activity involved the myenteric plexus,

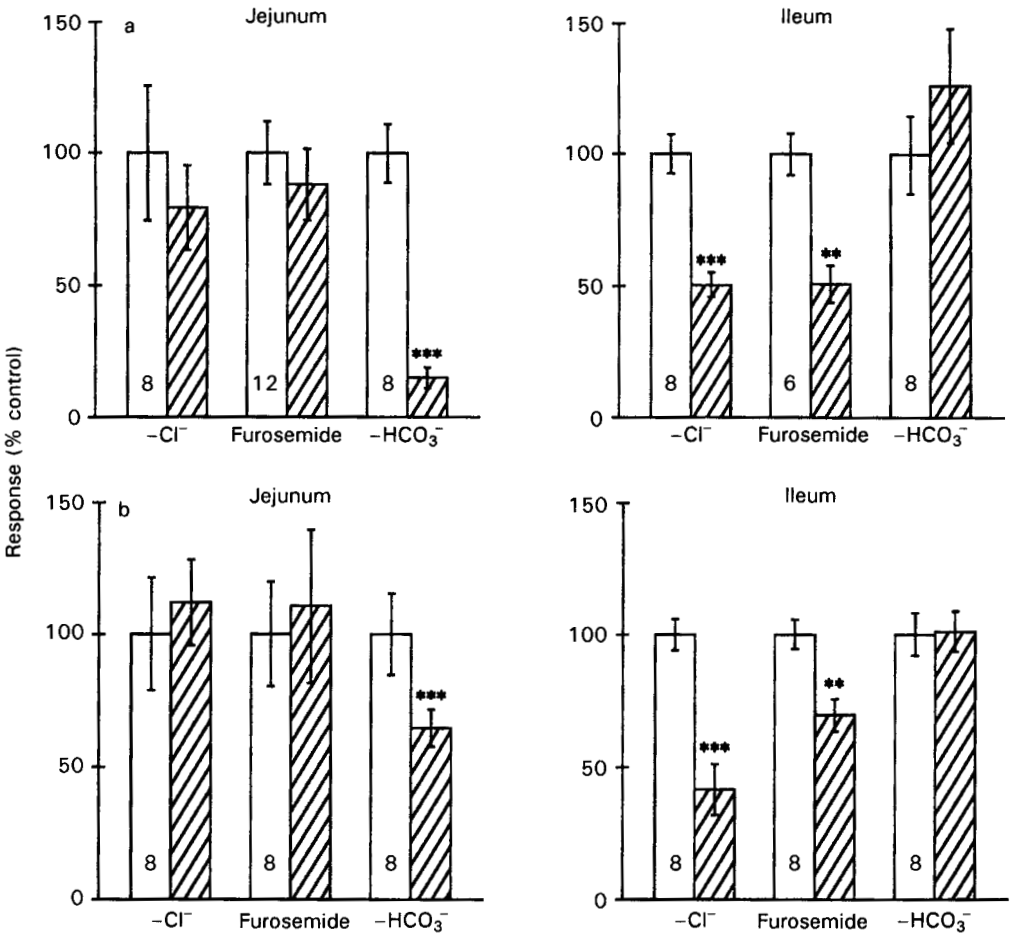


FIG. 2. Ionic basis of the response of intact (a) and stripped (b) sheets of rat jejunum and ileum to 100 $\mu$M 5-HT. Chloride was replaced with gluconate in the serosal solution ( $-Cl^-$ ) and bicarbonate with phosphate on both sides of the tissue ( $-HCO_3^-$ ). Furosemide was added to the serosal solution to give a final concentration of 1 mM, with control sheets receiving an equivalent volume (0·5% v/v) of the vehicle (dimethylsulphoxide). The increase in SCC induced by 5-HT is given as a percentage of control values. □ Control; ▨ test. 100% Values are $78{\cdot}9 \pm 7{\cdot}2$ $\mu$A cm$^{-2}$ (n = 28) and $28{\cdot}1 \pm 1{\cdot}9$ $\mu$A cm$^{-2}$ (n = 22) in intact preparations and $45{\cdot}8 \pm 5{\cdot}0$ $\mu$A cm$^{-2}$ (n = 24) and $56{\cdot}2 \pm 2{\cdot}9$ $\mu$A cm$^{-2}$ (n = 24) in stripped preparations of jejunum and ileum, respectively. Each bar represents the mean ± s.e.m. of the number of observations indicated; a paired $t$-test was used to assess significance. $^{**}P < 0{\cdot}01$; $^{***}P < 0{\cdot}001$.

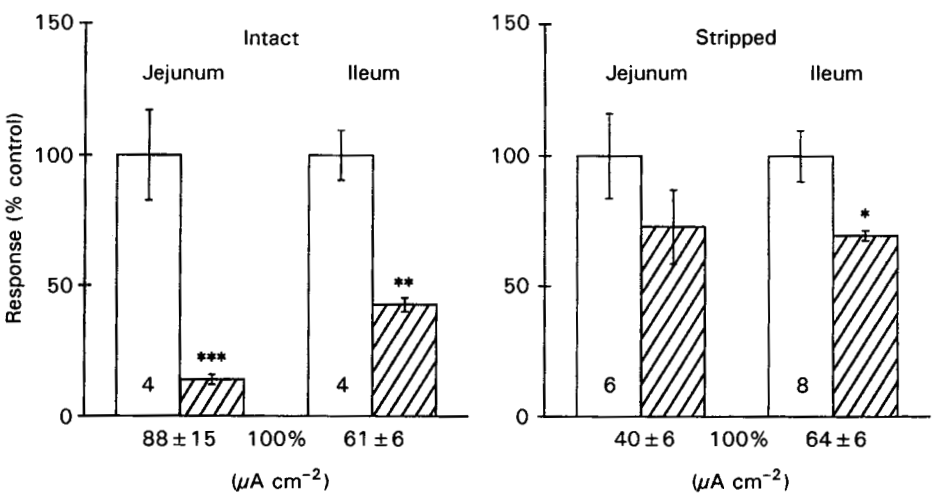


FIG. 3. Effect of serosal tetrodotoxin (10 $\mu$M) on the increase in SCC induced by 100 $\mu$M 5-HT in intact and stripped sheets of rat jejunum and ileum. The increase in SCC is given as a percentage of control values, with 100% values indicated. □ Control; ▨ plus tetrodotoxin. Each bar represents the mean ± s.e.m. of the number of observations indicated; a paired $t$-test was used to assess significance. $^{*}P < 0{\cdot}05$; $^{**}P < 0{\cdot}01$; $^{***}P < 0{\cdot}001$.

because tetrodotoxin had no significant effect on basal electrical activity in stripped jejunal sheets. Similar observations have been made in mouse jejunum (Sheldon et al 1989; Grubb 1995). That tetrodotoxin failed to inhibit basal SCC in jejunal sheets from mice with cystic fibrosis, which lack the ability to secrete chloride, suggests the existence of a neurally activated basal secretory tone in normal tissues (Grubb 1995). Tetrodotoxin did not alter basal SCC values in either preparation of rat ileum; this contrasts with the inhibition observed for intact and stripped sheets of guinea-pig and rabbit ileum (Hubel 1978; Baird & Cuthbert 1987; Smith et al 1990). This might, however, represent a species difference as other studies in the rat also failed to demonstrate a tetrodotoxin-sensitive component in basal SCC (Li et al 1994). In the presence of the neurotoxin basal SCC was still lower in the ileum than in the jejunum, suggesting that there must be a contribution from non-neural mechanisms. The simplest explanation is that the area of transporting tissue relative to the serosal area is lower in more distal regions of the small intestine (Levin et al 1983).

As well as differences in basal electrical activity, responses to 5-HT were also different in the jejunum and ileum. In intact preparations the maximum increase in SCC induced by 5-HT was similar in jejunum and ileum, but there was a far greater neural component in the jejunum where tetrodotoxin reduced the response to 5-HT by 86%, compared with 57% inhibition in the ileum. The myenteric plexus contributed to the neural processes involved in the jejunal response as its removal in the stripped preparation reduced by 58% the increase in SCC induced by a maximal concentration of 5-HT. In the ileum the myenteric plexus seemed to be less important. Stripping did not reduce the maximum SCC response to 5-HT, although the tetrodotoxin-induced inhibition of 5-HT action was reduced in the stripped preparation. This suggests some involvement of the myenteric plexus together with a contribution from other neural elements within the gut wall and is in agreement with results from other studies of guinea-pig (Cooke & Carey 1985; Baird & Cuthbert 1987) and rat (Castro et al 1987) small intestine.

In both the jejunum and ileum the residual response to 5-HT in the presence of tetrodotoxin was increased by stripping, although in the absence of the neurotoxin stripping reduced the response in the jejunum and had no effect in the ileum. It therefore appears that a non-neural (i.e. tetrodotoxin-insensitive) inhibitory mechanism is removed with the outer muscle layers and myenteric plexus. A possible candidate is nitric oxide which is produced not only by enteric neurones (Brookes 1993) but also by the enterocytes (Tepperman et al 1993) where its release is unlikely to be affected by tetrodotoxin. Several reports have suggested that nitric oxide has a pro-absorptive action in the intestine (Barry et al 1994; Schirgi-Degen & Beubler 1995), although other studies point to a pro-secretory role (Izzo et al 1994, 1996; Rhoads et al 1995; Kadowaki et al 1996). It is possible that the site of nitric oxide release might be important, with neural nitric oxide involved in stimulating secretion (Kadowaki et al 1996) whereas epithelial nitric oxide promotes absorption.

Differences between the jejunal and ileal responses to 5-HT are not confined to the mechanisms by which secretion is stimulated. In addition, different electrogenic ion transport processes are activated by 5-HT in these two regions of the intestinal tract. It has been established for many years that in mid and distal regions of the small intestine 5-HT induces chloride secretion (Ormsbee & Fondacaro 1985). However, a preliminary report by Urquhart et al (1988) suggested that in the jejunum bicarbonate, rather than chloride, was the anion secreted and this is supported by the current study. The reasons for this difference are not clear. It has been shown that bicarbonate ions can pass through the anion channels that open on secretory stimulation (Frizzell & Halm 1990) and if bicarbonate ions were present at a higher concentration in jejunal enterocytes they might preferentially leave the cell through the open anion channels. Mucosal carbonic anhydrase activity is greater in the jejunum than in the ileum (Charney et al 1986) and this could lead to higher intracellular bicarbonate levels in the more proximal regions of the small intestine.

5-HT-induced intestinal secretion is, therefore, a complex process, involving several different 5-HT receptor subtypes acting at different locations. This complexity is increased by the findings of the current study which indicate that in rat small intestine there are, in addition, regional differences in the nature of the response, with bicarbonate secretion predominating in the jejunum and chloride secretion in the ileum. Moreover, the balance between neural and non-neural components varies in these two regions. Such differences explain why there is yet no consensus about the mechanisms underlying the intestinal secretory response to 5-HT.

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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

Barry, M. K., Aloisi, J. D., Pickering, S. P., Yeo, C. J. (1994) Nitric oxide modulates water and electrolyte transport in the ileum. Ann. Surg. 219: 382–388

Beubler, E., Horina, G. (1990) 5-$HT_2$ and 5-$HT_3$ receptor subtypes mediate cholera toxin-induced intestinal fluid secretion in the rat. Gastroenterology 99: 83–89

Beubler, E., Coupar, I. M., Hardcastle, J., Hardcastle, P. T. (1990) Stimulatory effects of 5-hydroxytryptamine on fluid secretion and transmural potential difference in rat small intestine are mediated by different receptor subtypes. J. Pharm. Pharmacol. 42: 35–39

Beubler, E., Schirgi-Degen, A., Gamse, R. (1993) Inhibition of 5-hydroxytryptamine- and enterotoxin-induced fluid secretion by 5-HT receptor antagonists in the rat jejunum. Eur. J. Pharmacol. 248: 157–162

Borman, R. A., Burleigh, D. E. (1993) Evidence for the involvement of a 5-$HT_4$ receptor in the secretory response of the small intestine to 5-HT. Br. J. Pharmacol. 110: 927–928

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

Brookes, S. J. H. (1993) Neuronal nitric oxide in the gut. J. Gastroenterol. Hepatol. 8: 590–603

Budhoo, M. R., Kellum, J. M. (1994) The $5HT_4$ receptor mediates 5-hydroxytryptamine-induced rise in short-circuit current in the human jejunum in-vitro. Surgery 116: 396–400

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

Burleigh, D. E., Borman, R. A. (1993) Short-circuit current responses to 5-hydroxytryptamine in human ileal mucosa are mediated by a 5-$HT_4$ receptor. Eur. J. Pharmacol. 241: 125–128

Castro, G. A., Harari, Y., Russell, D. (1987) Mediators of anaphylaxis-induced ion transport changes in small intestine. Am. J. Physiol. 253: G540–G548

Charney, A. N., Wagner, J. D., Birnbaum, G. J., Johnstone, J. N. (1986) Functional role of carbonic anhydrase in intestinal electrolyte transport. Am. J. Physiol. 251: G682–G687

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

Fozard, J. R. (1987) 5-HT: the enigma variations. Trends Pharmacol. Sci. 8: 501–506

Franks, C. M., Hardcastle, J., Hardcastle, P. T. (1996) Neural involvement in 5-hydroxytryptamine-induced electrogenic anion secretion in the rat intestine in-vivo. J. Pharm. Pharmacol. 48: 411–416

Frizzell, R. A., Halm, D. R. (1990) Chloride channels in epithelial cells. Curr. Top. Membr. Transport 37: 247–282

Grondahl, M. L., Hansen, M. B., Larsen, I. E., Skadhauge, E. (1996) Age and segmental differences in 5-hydroxytryptamine-induced hypersecretion in the pig small intestine. J. Comp. Physiol. B 166: 21–29

Grubb, B. R. (1995) Ion transport across the jejunum in normal and cystic fibrosis mice. Am. J. Physiol. 268: G505–G513

Hardcastle, J., Hardcastle, P. T. (1991) Increased transintestinal electrical activity of rat small intestine *in vivo* and *in vitro* induced by 5-methoxytryptamine. J. Physiol. 438: 364P

Hardcastle, J., Hardcastle, P. T. (1995) Evidence that the secretory response of rat intestine to 5-hydroxytryptamine in-vivo involves more than one 5-hydroxytryptamine receptor subtype. J. Pharm. Pharmacol. 47: 744–749

Hardcastle, J., Hardcastle, P. T. (1996a) Rat ileal response to 5-hydroxytryptamine (5-HT) *in vitro* involves more than one 5-HT receptor subtype. Physiol. Res. 45: 7P

Hardcastle, J., Hardcastle, P. T. (1996b) Comparison of the secretory response to 5-hydroxytryptamine (5-HT) in the jejunum and ileum of the rat *in vitro*. Physiol. Res. 45: 7P

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

Hendriks, R., Bornstein, J. C., Furness, J. B. (1989) Evidence for two types of 5-hydroxytryptamine receptor on secretomotor neurons of the guinea-pig ileum. Naunyn Schmiedebergs Arch. Pharmacol. 339: 409–414

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. Rec. 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

Hubel, K. (1978) The effects of electrical field stimulation and tetrodotoxin on ion transport by the isolated rabbit ileum. J. Clin. Invest. 62: 1039–1047

Izzo, A. A., Mascolo, N., Di Carlo, G., Capasso, F. (1994) $N^G$-nitro-L-arginine methyl ester modulates intestinal secretion and motility produced by carbachol. Eur. J. Pharmacol. 271: 31–35

Izzo, A. A., Gaginella, T. S., Mascolo, N., Borrelli, F., Capasso, F. (1996) $N^G$-nitro-L-arginine methyl ester reduces senna- and cascara-induced diarrhoea and fluid secretion in the rat. Eur. J. Pharmacol. 301: 137–142

Kadowaki, M., Gershon, M. D., Kuwahara, A. (1996) Is nitric oxide involved in 5-HT-induced fluid secretion in the gut? Behav. Brain Res. 73: 293–296

Levin, R. J., Mitchell, M. A., Barber, D. C. (1983) Comparison of jejunal and ileal absorptive functions for glucose and valine *in vivo* – a technique for estimating real $K_m$ and $J_{max}$ in the domestic fowl. Comp. Biochem. Physiol. 74A: 961–966

Li, Y. F., Weisbrodt, N. W., Lodato, R. F., Moody, F. G. (1994) Nitric oxide is involved in muscle relaxation but not in changes in short-circuit current in rat ileum. Am. J. Physiol. 266: G554–G559

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

Ormsbee, H. S., Fondacaro, J. D. (1985) Action of serotonin on the gastrointestinal tract. Proc. Soc. Exp. Biol. Med. 178: 333–338

Rhoads, J. M., Argenzio, R. A., Chen, W., Gomez, G. G. (1995) Asparagine stimulates piglet intestinal $Cl^-$ secretion by a mechanism requiring a submucosal glutamate receptor and nitric oxide. J. Pharmacol. Exp. Ther. 274: 404–412

Schirgi-Degen, A., Beubler, E. (1995) Significance of nitric oxide in the stimulation of intestinal fluid absorption in the rat jejunum *in vivo*. Br. J. Pharmacol. 114: 13–18

Schultz, S. G., Frizzell, R. A., Nellans, H. N. (1974) Ion transport by mammalian small intestine. Ann. Rev. Physiol. 36: 51–91

Scott, C. M., Bunce, K. T., Spraggs, C. F. (1992) Investigation of the 5-hydroxytryptamine receptor mediating the 'maintained' short-circuit current response in guinea-pig ileal mucosa. Br. J. Pharmacol. 106: 877–882

Sheldon, R. J., Malarchik, M. E., Fox, D. A., Burks, T. F., Porreca, F. (1989) Pharmacological characterization of neural mechanisms regulating mucosal ion transport in mouse jejunum. J. Pharmacol. Exp. Ther. 249: 572–582

Siriwardena, A., Kellum, J. M. (1993) A 5-$HT_2$ receptor mediates serotonin-induced electrolyte transport in rat left colon. J. Surg. Res. 55: 323–329

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

Smith, P. L., Chiossone, D. C., McCafferty, G. P. (1990) Characterization of $LTC_4$ effects on rabbit ileal mucosa in vitro. Naunyn Schmiedebergs Arch. Pharmacol. 341: 94–100

Tepperman, B. L., Brown, J. F., Whittle, B. J. R. (1993) Nitric oxide synthase induction and epithelial cell viability in rats. Am. J. Physiol. 265: G214–G218

Urquhart, C. J., Wilson, K. A., Downing, O. A., Roach, A. G., Lord, J. A. H. (1988) Effect of inhibitors of ion transport on 5-HT-induced increases in short circuit current in rat isolated jejunum. Br. J. Pharmacol. 94: 44P