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Short communication

# Human colonic mucosa possesses a mixed population of 5-HT receptors

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**Abstract**

The aim of this study was to characterise the 5-HT receptor(s) mediating secretory responses of isolated human colonic mucosa to 5-HT. Sheets of muscle-stripped mucosa from proximal (ascending) and distal (sigmoid) human colon were set up in Ussing chambers for measurement of short-circuit current ($I_{sc}$). Serosal application of 5-HT led to non-neural, concentration-dependent increases in $I_{sc}$. Desensitisation to 5-HT was observed with ascending and sigmoid colonic mucosa. Using selective 5-HT antagonists we have shown that 5-HT induces secretion in sigmoid colon via a 5-$HT_{2A}$ receptor. In ascending colon a combination of 5-$HT_{2A}$ and 5-$HT_4$ receptors appears to be involved.

*Keywords:* 5-HT (5-hydroxytryptamine, serotonin); 5-HT receptor; Secretion; (Human); Colon; Mucosa

## 1. Introduction

Examination of the receptor type mediating the secretory effects of 5-hydroxytryptamine (5-HT) in mammalian colon has produced variable results. In rat colonic mucosa, Bunce et al. (1991) provided evidence for existence of 5-$HT_4$ receptors whereas Siriwardena et al. (1991) reported evidence for involvement of 5-$HT_2$ receptors. In guinea-pig colon, Cooke et al. (1991) have shown 5-$HT_3$ receptors to mediate the secretory effects of 5-HT. More recently Ayton et al. (1995) have shown a segmental heterogeneity of the receptors mediating responses of rat colon to 5-HT. In the proximal region, 5-$HT_3$ receptors mediate secretory responses to 5-HT whereas distally the secretory response is due to activation of more than one type of receptor. The present study attempts to determine the receptor(s) responsible for 5-HT-induced fluid secretion in human colonic mucosa. A preliminary report of this work has been made to the British Pharmacological Society (Borman and Burleigh, 1996).

## 2. Materials and methods

### 2.1. Setting up of mucosal preparations

Sigmoid colon was obtained at operations for carcinoma of sigmoid colon or rectum, ascending colon was obtained at operations for carcinoma of caecum or ascending colon. The methods have been described previously (Borman and Burleigh, 1993). Tissue preparations were allowed to equilibrate for 60 min under short-circuit conditions before addition of drugs. At 5 min intervals, the tissue was clamped at 2 mV (rather than 0 mV) and conductance calculated from the resulting change in short-circuit current ($I_{sc}$). Forskolin, 5-HT and 5-carboxamidotryptamine were applied to the serosal side, 5-HT receptor antagonists and tetrodotoxin to both sides of the preparation.

### 2.2. Statistical analysis

Responses to 5-HT were expressed as a percentage of the maximum $I_{sc}$ response to forskolin (25 $\mu$M). $EC_{50}$ values for 5-HT were determined graphically from individual concentration-response curves. Concentration ratios were calculated from $EC_{50}$ values and expressed as geometric mean with 95% confidence limits; all other data are given as arithmetic mean $\pm$ S.E.M. In all cases, $n$ indicates the number of patients studied. Statistical comparisons used the Mann-Whitney $U$ test, with $P < 0.05$ being taken to represent a significant difference.

### 2.3. Drugs

Drugs used were: 5-carboxamidotryptamine maleate (RBI), 1-*H*-benzimidazole-1-carboxylic acid, 2,3-dihydro-

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6-methoxy-2-oxo-8-methyl-8-azabicyclo(3,2,1)oct-3-yl ester chloridate (DAU-6285; Boehringer Ingelheim, Italy); forskolin, tetrodotoxin, 5-hydroxytryptamine creatinine sulphate (all Sigma); ketanserin tartrate (Janssen); methysergide hydrogen maleate (Sandoz); ondansetron hydrochloride (Glaxo).

## 3. Results

### 3.1. Ascending colon

For ascending colon, after 60 min equilibration, basal $I_{sc}$ was $65.6 \pm 6.2$ $\mu$A cm$^{-2}$, and tissue conductance was $9.5 \pm 1.9$ mS cm$^{-2}$ ($n = 14$). 5-HT (1–100 $\mu$M), applied cumulatively with a 3 min contact time (sufficient for each concentration to achieve its maximal effect), gave concentration-dependent increases in $I_{sc}$ (Fig. 1). A maximal response of $32.4 \pm 2.5$ $\mu$A cm$^{-2}$ was achieved with an $EC_{50}$ of 3.74 $\mu$M (95% confidence limits, 3.0–4.7, $n = 7$). There was significant desensitization for a second concentration-response curve to 5-HT with depression of the maximum response to 5-HT. It was therefore decided to obtain just a single concentration-response curve to 5-HT in any one preparation, either in the absence or presence of antagonist, which was added 30 min prior to challenge with 5-HT.

Tetrodotoxin (TTX; 3.1 $\mu$M), methysergide (10 $\mu$M) or ondansetron (10 $\mu$M, all $n = 6$), had no effect on the secretory response of ascending colonic mucosa to 5-HT. However, application of DAU 6285 (10 $\mu$M) caused a rightward displacement of the concentration-response curve to 5-HT in 4 out of 6 specimens (Fig. 1), with no alteration of the maximum response to 5-HT, yielding a single concentration $pA_2$ estimate (Mackay, 1978) in those 4 specimens of $5.78 \pm 0.21$. In the remaining 2 specimens, the response to 5-HT was shown to be insensitive to prior application of DAU 6285; however, application of ketanserin (1 $\mu$M) was shown to abolish the $I_{sc}$ response to 5-HT. This concentration of ketanserin had no effect on the secretory response to 5-HT in the 4 specimens which were sensitive to DAU 6285. DAU 6285 and ketanserin had no significant effect on basal $I_{sc}$ ($n = 6$, $P > 0.05$).

### 3.2. Sigmoid colon

For sigmoid colon, after 60 min equilibration, basal $I_{sc}$ was $71.3 \pm 2.8$ $\mu$A cm$^{-2}$, and tissue conductance was $7.5 \pm 0.6$ mS cm$^{-2}$ ($n = 82$). For both ascending and sigmoid colonic mucosa, tissue conductance did not alter significantly throughout the experiment. Nearly one-third (29%) of sigmoid colonic preparations failed to respond to 5-HT. Of those which did respond nearly half (43%) failed to elicit a second response on repetition of the challenge with 5-HT. For this reason a protocol was adopted whereby each preparation from a specimen received just a single, different concentration of 5-HT, either in the absence or presence of antagonist.

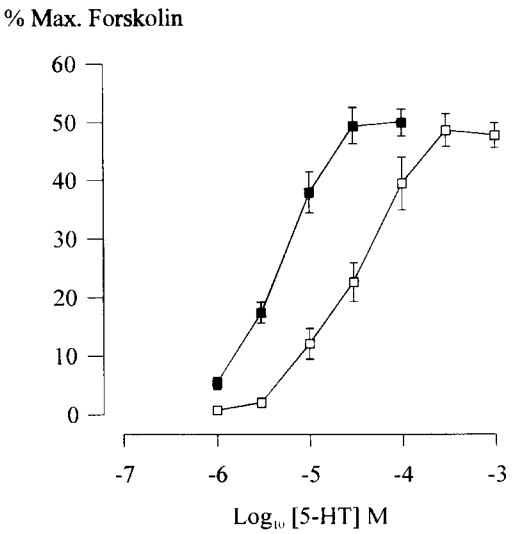


Fig. 1. Effect of DAU 6285 on the $I_{sc}$ response to 5-HT of human ascending colonic mucosa. Figure shows the concentration-response curve to 5-HT either in the absence (■) or presence of DAU 6285 at a concentration of 10 $\mu$M (□). Data are expressed as percentage of the maximum $I_{sc}$ secretory response to forskolin (25 $\mu$M) and are given as mean ± S.E.M. for $n = 4$ out of 6 tissues. In two further tissues, the response was unaffected by prior application of DAU 6285.

An additional problem with sigmoid colon was the influence of warm ischaemia. Warm ischaemia is the time between the occlusion of the main blood vessels in situ and the removal of the specimen from the patient. It is brief (typically 5–10 min) for right-sided resections, from which ileal and ascending colonic mucosa were obtained.

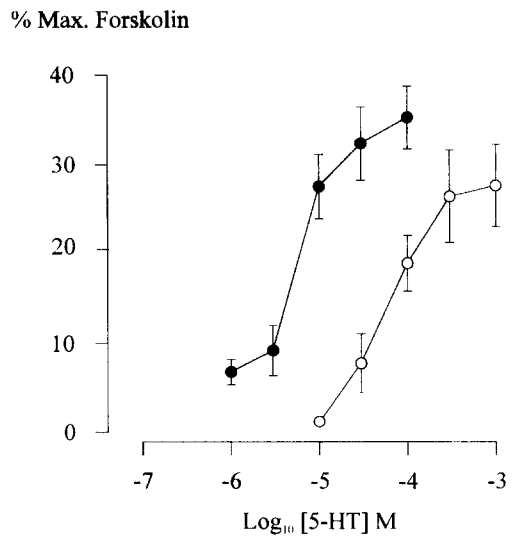


Fig. 2. Effect of ketanserin on the $I_{sc}$ response to 5-HT of human sigmoid colonic mucosa. Figure shows concentration-response curve to 5-HT either in the absence (●) or presence of ketanserin at a concentration of 30 nM (○), where each individual preparation is challenged by just a single concentration of 5-HT, either in the absence or presence of antagonist. Data are expressed as a percentage of the maximum secretory response to forskolin (25 $\mu$M) and are given as mean ± S.E.M. for $n \geq 4$.

For left-sided colonic resections it frequently approached 30–40 min which adversely affected sensitivity to 5-HT. Maximal secretory responses to 5-HT (30 $\mu$M) were attained when ischaemia was reduced to less than 5 min. When ischaemic time was increased by a further 5 min, there was a significant reduction of the $I_{sc}$ response to 5-HT ($10.0 \pm 2.9$ compared to $17.8 \pm 2.4$ $\mu$A cm$^{-2}$, $n =$ 10 and 20 respectively $P < 0.05$), a trend which continued up to 45 min of ischaemia. Responses to electrical field stimulation and forskolin were not significantly reduced, even when warm ischaemic time was in excess of 30 min ($n = 12$, data not shown). On this basis, further experiments were only performed when ischaemia was 5 min or less.

5-HT (1 $\mu$M–1 mM) gave a concentration-dependent increase in $I_{sc}$, which was maximal at 100 $\mu$M 5-HT (Fig. 2) giving a maximum response of $35.0 \pm 3.0$ $\mu$A cm$^{-2}$ with an $EC_{50}$ of 5.3 $\mu$M (95% confidence limits 2.8–9.1; $n \geq 4$).

It was decided initially to test the efficacy of antagonists against a single dose of 5-HT, namely 10 $\mu$M, which was an approximate $EC_{75}$ value for 5-HT. Application of TTX (3.1 $\mu$M), ondansetron (10 $\mu$M) or DAU 6285 (10 $\mu$M, all $n = 4$) had no significant effect on the secretory response of human sigmoid colonic mucosa to 5-HT. In contrast, application of ketanserin (1 $\mu$M) was shown to abolish the $I_{sc}$ response to 5-HT while having no significant effect on basal $I_{sc}$ ($n = 4$, $P > 0.05$). The effects of ketanserin were further investigated across a range of concentrations of 5-HT. Application of 30 nM ketanserin was shown to cause a rightward displacement of the concentration-response curve to 5-HT (Fig. 2). There appeared to be a reduction of the maximum response to 5-HT but as this trend failed to reach statistical significance a single-concentration $pA_2$ estimate of $8.5 \pm 1.1$ ($n = 4$) was calculated.

Application of the potent and selective 5-HT$_1$ receptor agonist, 5-carboxyamidotryptamine (10 $\mu$M), produced no significant change in basal $I_{sc}$ ($n = 3$, $P > 0.05$), this being in tissues which subsequently responded to 5-HT.

## 4. Discussion

The $EC_{50}$ values for 5-HT on human colonic mucosa are similar to those obtained from rat colonic and human small intestinal mucosa (Bunce et al., 1991; Borman and Burleigh, 1993; Kellum et al., 1994) although the maximum responses were lower. Coincidentally, cultured human colonic epithelial cells ($T_{84}$) lack sensitivity to 5-HT while responding to other secretagogues (Dharmsathaphorn et al., 1984). The secretory action of 5-HT on human colonic mucosa may be important in contributing to diarrhoea of the carcinoid syndrome. A $pA_2$ estimate of 5.78 for DAU 6285 is lower than has been reported for interaction at the 5-HT$_4$ receptor in guinea-pig ileum (6.88; Schiavone et al., 1992), but is only slightly lower than the value found in human ileal mucosa (6.17; Borman and Burleigh, 1993) and that reported at the 5-HT$_4$-like receptor in human colonic smooth muscle (6.3; Tam et al., 1995). Indeed, Tam made note of the fact that DAU 6285 seems to exhibit a $pK_B$ value which is somewhat lower in humans than in animal studies, and that this may indicate a species difference between the receptors termed 5-HT$_4$ in human and animal tissues. In two further specimens of ascending colon, the antagonist profile observed more closely resembles an interaction with a receptor of the 5-HT$_{2A}$ subtype, rather than a 5-HT$_4$ receptor. This may not be totally unexpected when we consider the response of human sigmoid colonic mucosa to 5-HT. Here, 5-HT was shown to induce fluid secretion via a receptor which was sensitive to prior application of ketanserin, yielding a single-concentration $pA_2$ estimate of 8.5. Ketanserin is a selective antagonist for the 5-HT$_{2A}$ (formerly known as 5-HT$_2$) receptor, with affinity estimates of 8.5–9.5 (Humphrey et al., 1993). It would therefore seem likely that the receptor mediating the secretory response to 5-HT changes down the human gastrointestinal tract, from a 5-HT$_4$ receptor in the small intestine, to a 5-HT$_{2A}$ receptor in the sigmoid colon, with the ascending colon denoting the convergence of these two receptor subtypes.

Investigation of the causes underlying desensitisation was beyond the scope of this study. Desensitisation of the 5-HT$_2$ receptor has been previously encountered, and comprehensively investigated, in rat cortical neurones (Rahman and Neuman, 1993). The reason why prolonged 'warm ischaemic' time leads to a reduced response to 5-HT is unknown.

In summary, 5-HT induces a non-neural electrogenic fluid secretion in both ascending and sigmoid colonic mucosa, albeit to a lesser magnitude than that evoked in human ileal mucosa (Borman and Burleigh, 1993). The receptor responsible for these effects differs between regions. Such regional variations have also been observed in rat colon (Ayton et al., 1995). We have shown that 5-HT induces secretion in sigmoid colon via a 5-HT$_{2A}$ receptor, whereas the response in ascending colon cannot be definitively classified at this time, but may reflect the presence of a heterogenous population of 5-HT$_{2A}$ and 5-HT$_4$ receptors.

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