Inhibition of the effect of serotonin on rat ileal transport by cisapride: evidence in favour of the involvement of 5-HT$_2$ receptors

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SUMMARY Cisapride is a synthetic drug which binds, *in vitro*, to type 2 serotonin receptors. We examined the influence of serotonin and cisapride on ion transport across intestinal mucosa *in vitro* and studied the effect of cisapride on the response to serotonin. Segments of ileum of male Sprague-Dawley rats were stripped of muscle layers and mounted in flux chambers. The addition of serotonin (10^{-8} to 10^{-4}M) to the serosal aspect of the mucosa caused a rapid, dose-dependent rise in short circuit current and transmural potential difference. Cisapride alone (5×10^{-5}M), when added to the mucosal and serosal surfaces, had no effect on the short circuit current, transmural potential difference, resistance, or sodium and chloride fluxes across the mucosa. It did, however, inhibit the response of the mucosa to serotonin (10^{-5}M) in a dose dependent manner and blocked it completely at a concentration of 5×10^{-5}M. Serotonin (5×10^{-5}M) increased serosal to mucosal flux of chloride from $12 \cdot 6 \pm 0 \cdot 8$ to $15 \cdot 2 \pm 0 \cdot 6$ µmol/cm^2/h (p<0·025), thus reducing net chloride absorption from $4 \cdot 65 \pm 0 \cdot 81$ to $1 \cdot 49 \pm 1 \cdot 04$ µmol/cm^2/h (p<0·05). This effect was completely blocked by cisapride (5×10^{-5}M). In summary, cisapride inhibits the effect of serotonin on rat ileal ion transport, probably by blocking type 2 serotonin receptors.

Serotonin (5-HT) is found in high concentrations throughout the gastrointestinal tract of a variety of mammalian species including man.[1] In the gut, the amine is contained predominantly within the mucosal enterochromaffin cells,[2] but it is also found in the myenteric plexus,[3-5] suggesting a neurotransmitter role for 5-HT in the gut.[6]

Serotonin stimulates fluid secretion across a variety of mammalian gastrointestinal epithelia, including rat jejunum[7] and colon[8 9] and rabbit gall bladder, colon, and ileum.[10-15] It is uncertain, however, whether these effects are mediated *via* 5-HT$_1$ or 5-HT$_2$ types of receptor, largely because of a lack of good specific 5-HT blocking agents.

Cisapride (Janssen Pharmaceutica, Beerse, Belgium) is a recently developed drug which stimulates gastrointestinal motor activity in a number of

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mammalian species including man.[16] It binds specifically to type 2 serotonin receptors *in vitro* having no affinity for type 1 serotonin binding sites,[17] and it antagonises contractions induced by serotonin in the guinea pig ileum and the tail artery of the rat.[18] The aim of the present study was to examine whether this relatively specific 5-HT$_2$ receptor antagonist would help define the type of receptor likely to be involved in mediating the intestinal mucosal response to 5-HT.

Methods

ANIMALS
Non-fasting male Sprague-Dawley rats were killed by a blow to the head and cervical dislocation. The ileum was removed and stripped of muscle layers. Segments of mucosa were mounted between perspex flux chambers with a surface area of 0·64 cm^2. Usually eight tissues from the same animal were mounted so that luminal and basal aspects were each bathed in buffer solution containing Na, 146; K, 4·2; Cl, 125·8;

HCO_3, 26·6; H_2PO_4, 0·2; HPO_4, 1·2; Ca, 1·2; Mg, 1·2; glucose, 10 (all mmol/l); pH 7·4 at 37°C. All solutions were continuously oxygenated with 95% O_2 – 5% CO_2.

ELECTRICAL MEASUREMENTS

The spontaneous transmucosal electrical potential difference (PD) was measured *via* 3M KCl in 3% agar electrode bridges and matched calomel half cells to a high impedance digital voltmeter. Short circuit current (SCC) was delivered *via* silver/silver chloride electrodes and 1M NaCl in 1% agar bridges. The electrodes were connected to a voltage clamp for automatic short-circuiting, the clamp being corrected for the fluid resistance between the PD sensing bridges. Tissue resistance (R) was calculated from PD and SCC according to Ohm's law and the SCC in μamps was converted to net ionic fluxes as previously described.[19]

RADIO-ISOTOPE FLUXES

Isotopic fluxes were measured after adding 0·5 μCi ^{22}Na and 2·5 μCi ^{36}Cl (Radiochemical Centre, Amersham, Bucks, England) to the mucosal side of one tissue and the serosal side of its paired tissue. Tissues were paired providing their resistances did not differ by more than 25%. The method for determining and calculating the unidirectional fluxes has been described.[19] The net flux was calculated as the difference between the two unidirectional fluxes. In the flux experiments, isotopes were added immediately after the tissue was mounted. After a 20 minute equilibration period, five consecutive 15 minute fluxes were determined.

CHEMICALS

Cisapride was supplied by Janssen Pharmaceuticals. It was dissolved in a base containing mannitol and acetic acid. In all experiments, cisapride or the base were added simultaneously to both the mucosal and serosal chambers, 15 minutes before the addition of serotonin. Serotonin (Sigma Chemical Co, St Louis, Missouri, USA) was added to the serosal chamber only.

CALCULATIONS

Residual ion flux, which is that part of the SCC in μmol/cm²/h not accounted for by net movement of Na and Cl, was calculated as SCC – (JNa net – JCl net).

Statistical comparisons were carried out by the Student's *t* test.[20]

Results

ELECTRICAL EXPERIMENTS

Serotonin, when added to the serosal aspect of the mucosa, in concentrations ranging from 10^{-8} to $10^{-4}M$, caused a rapid, dose dependent rise in short circuit current (Fig. 1) and transmucosal electrical potential difference. This response was maximal 60 seconds after the addition of 5-HT and lasted from 15–30 minutes. Transmural resistance was unaltered.

The addition of either cisapride, in concentrations up to $5 \times 10^{-5}M$, or its base, to the mucosal and serosal aspects of the mucosa had no effect on the short circuit current, transmural potential difference or resistance.

The application of serotonin ($10^{-5}M$) to the serosal aspect of the mucosa caused a rapid increase in short-circuit current and potential difference. Cisapride, however, inhibited this response in a dose dependent manner and blocked it completely at a concentration of $5 \times 10^{-5}M$ (Fig. 2).

FLUX EXPERIMENTS

The influence of serotonin ($5 \times 10^{-5}M$) on net and unidirectional chloride fluxes in the presence and absence of cisapride is shown in Figure 3. Cisapride itself had no effect on net or unidirectional fluxes of
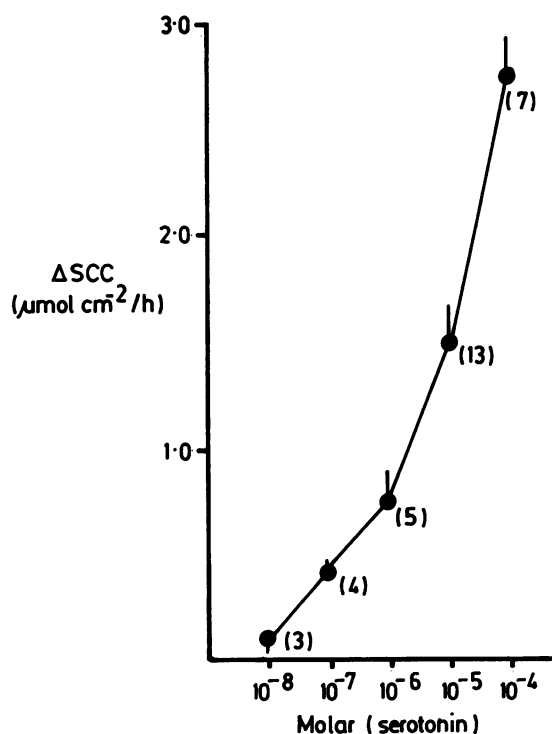
Fig. 1 *Influence of serotonin on the peak increments in short circuit current (ΔSCC). Results are the mean±SEM. Numbers in brackets represent the numbers of tissues studied at each concentration.*
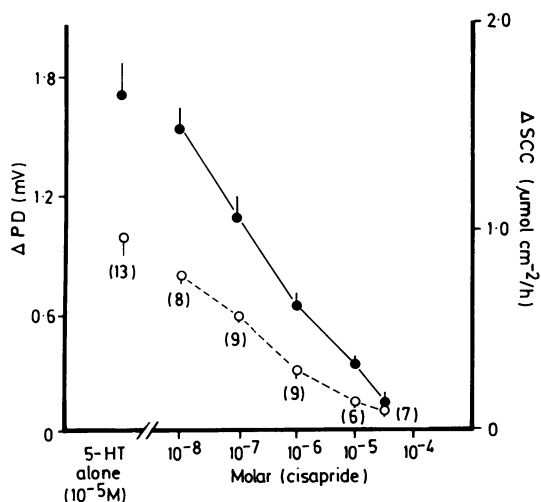
Fig. 2 *Influence of increasing concentrations of cisapride on the peak increments in short circuit current (solid line) and transmural electrical potential difference (dashed line), which occurred in response to $10^{-5}M$ serotonin (5-HT). Cisapride was added 15 mins before serotonin. Numbers in brackets represent the number of tissues studied at each concentration. Results are expressed as the mean±SEM.*

sodium or chloride or on residual ion flux. In contrast, serotonin increased serosal to mucosal flux of chloride from 12·6±0·8 to 15·2±0·6 μmol/cm²/h (p<0·025), thus reducing net chloride absorption from 4·65±0·81 to 1·49±1·04 μmol/cm²/h (p<0·05). This effect was completely blocked by cisapride. This change in chloride flux lasted for 15 minutes only (Fig. 3) and corresponded to the time course of the rise in short circuit current and transmucosal electrical potential difference which were observed in response to the secretagogue. Serotonin had no effect on net or unidirectional fluxes of sodium, nor did it have any effect on residual ion flux.

Discussion

Several studies have shown that serotonin can cause secretion in rat jejunal[7] and colonic[8 9] mucosa and rabbit ileal and colonic[10-15] mucosa. The present studies in rat ileum confirm that 5-HT increases serosal to mucosal fluxes of chloride, thereby inhibiting net absorption. In other studies, mucosal to serosal fluxes of sodium and chloride secretion was thus induced by 5-HT and chloride secretion was thus induced. Differences in species, in region of intestine studied or in dosage of 5-HT used might account for this discrepancy.

The mechanism of action of 5-HT involves a rise in intracellular calcium,[12-15] but the site and type of serotonin receptor which mediates the response is

uncertain. Attempts to show specific binding receptors on epithelial cells from rat intestine[21] were unsuccessful. On the other hand, if a neural intermediary was involved in the serotonin effects, it would be expected that the nerve blocking agent, tetrodotoxin, would have inhibited responses to 5-HT, but this could not be demonstrated, at least in rat colon.[9]

Whatever the site of 5-HT receptors, the type involved in influencing transport has not been clearly defined, largely because of a lack of specific 5-HT blockers. Serotonin receptors in the brain are classified as either type 1, which have a high affinity for 5-HT and a low affinity for spiroperidol, or type 2, which have a lower affinity for 5-HT and a higher affinity for spiroperidol.[22 23] In the rat colon, however, neither cinanserin, a potent type 2 antagonist,[24] nor methysergide, which has a relatively high affinity for type 1 and type 2 receptors, had any effect on the rise in short circuit current induced by 5-HT.[9] In contrast, in the rat jejunum, mianserin, a potent type 2 antagonist, but which also possesses weak affinity for type 1 serotonin binding sites, competitively inhibited the response to 5-HT.[7] The binding affinity of cisapride is moderate for type 2 serotonin receptors, weak for α_1-adrenergic, absent for serotonin type 1, and virtually absent for dopamine-2, histamine-1, and muscarinic receptors.[17] In the present study, cisapride blocked the inhibition of chloride absorption caused by 5-HT. In addition, as in guinea pig ileum and rat colon,[25] cisapride inhibited the rise in short circuit current and transmucosal electrical potential difference which occurred in response to 5-HT. These findings suggest that 5-HT acts *via* type 2 serotonin receptors in stripped rat ileum.

In the present study, cisapride itself had no influence on electrical activity or ion fluxes. This and previous similar reports[26 27] suggest that endogenous serotonin does not contribute to the intrinsic transport 'tone' of the stripped rat ileal preparation.

The precise physiological role of 5-HT is unknown, but it is thought that its release postprandially leads to simultaneous increases in intestinal motility, secretion and blood flow, thus facilitating the digestive and absorptive functions of the gut.

Pathophysiologically, raised plasma concentrations of 5-HT are usually the cause of the secretory diarrhoea characteristic of the carcinoid syndrome.[28 29] The findings of the present study would suggest that cisapride may be useful in patients with this syndrome, although its effect on intestinal motility in this condition is unpredictable.
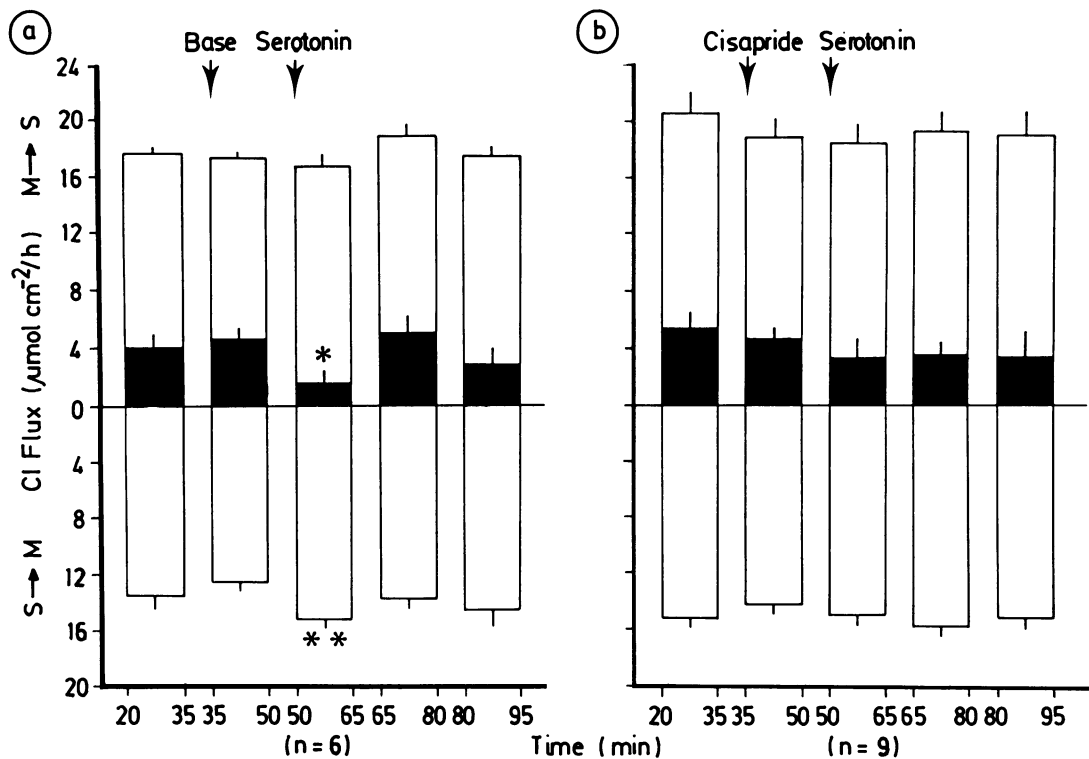
Fig. 3 *Influence of serotonin ($5 \times 10^{-5}M$) on net (shaded bars) and unidirectional chloride fluxes in the presence of the base (a) and $5 \times 10^{-5}M$ cisapride (b), $m \rightarrow s$ is mucosal to serosal flux, $s \rightarrow m$ is serosal to mucosal flux. Results are the mean $\pm$ SEM. *$p<0\cdot05$, **$p<0\cdot025$. Values refer to the level of significance of the differences between the second and third flux periods.*

financial support and to Julie Rostron for typing the manuscript.

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