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Stimulatory Effects of 5-Hydroxytryptamine on Fluid Secretion and Transmural Potential Difference in Rat Small Intestine are Mediated by Different Receptor Subtypes

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Abstract—The rise in transmural potential difference (PD) and the fluid secretion induced by 5-hydroxytryptamine (5-HT) were measured in rat small intestine in-vivo. Both cisapride and ketanserin abolished the 5-HT-induced rise in systolic blood pressure mediated by 5-HT_2 receptors. Cisapride inhibited the 5-HT-induced increases in the transintestinal PD, but over the same dose range it had no effect on the fluid secretion induced by 5-HT. In contrast, ketanserin caused a dose-dependent reduction in 5-HT-induced fluid secretion at doses that failed to influence the rise in PD. It is concluded that different receptors are responsible for the effects of 5-HT on fluid secretion and electrical activity in the rat small intestine.

The ability of 5-hydroxytryptamine (5-HT) to influence transport processes in the small intestine is well-established. In this tissue 5-HT causes a net secretion of fluid and electrolytes into the intestinal lumen (Kisloff & Moore 1976; Donowitz et al 1977) and flux determinations have shown that a decrease in coupled NaCl absorption and a stimulation of net Cl^- secretion could be responsible (Donowitz et al 1980; Hardcastle et al 1981). Since the Cl^- secretory mechanism is electrogenic, 5-HT action is associated with a rise in the potential difference and short-circuit current across the intestinal wall (Hardcastle et al 1981). The nature of the 5-HT receptors that mediate the stimulation of Cl^- secretion and inhibition of NaCl absorption has not yet been clearly established and most studies have used the 5-HT-induced rise in transintestinal electrical activity to monitor the effects of antagonists on 5-HT action on intestinal ion transport (Cooke & Carey 1985; Baird & Cuthbert 1987; Moriarty et al 1987; Bunce et al 1988). Such methods do not however, detect changes in the activity of electrically neutral ion transport processes, so in this study the effect of 5-HT was measured both as a change in electrical activity and a stimulation of fluid secretion. The effects of two 5-HT antagonists on these actions of 5-HT were investigated. Both ketanserin (Bradley et al 1986) and cisapride (Schuurkes & Van Nueten 1985) have been described as 5-HT_2 antagonists, with cisapride also exhibiting weak 5-HT_3 antagonism (Dunbar et al 1986; Van Nueten & Schuurkes 1989). In this study however, ketanserin and cisapride, in spite of their both demonstrating 5-HT_2 antagonism, exerted different effects on 5-HT-induced changes in fluid movement and electrical activity in the small intestine.

Materials and Methods

Measurement of transintestinal potential difference in-vivo

Experiments were carried out on male albino rats, 230–250 g, obtained from the Sheffield Field Laboratories and allowed free access to food (diet 86, Oxoid, London) and water. The rats were anaesthetized with sodium pentobarbitone (60 mg kg^{-1} i.p.).

The transintestinal potential difference (PD) was measured in-vivo across a 5 cm segment of mid-intestine. This was tied off at the distal end and a cannula was inserted into the proximal end. The loop and the peritoneal cavity were then filled with 154 mM NaCl. The PD was measured between two salt bridge electrodes, one in contact with the luminal fluid and the other in contact, via a wick electrode, with the fluid in the peritoneal cavity. These electrodes were connected via calomel half cells to a Vibron electrometer (Electronic Instruments Ltd., model 33B-2) whose output was displayed on a Vitatron chart recorder (MSE Scientific Instruments, 2001 series). Drugs were administered through a cannula in the jugular vein and each dose was washed in with 0·2 mL 154 mM NaCl. The electrical response to 5-HT was taken as the difference between the maximum PD achieved after each dose of the drug and the value immediately before its addition. A dose-response curve was constructed for 5-HT before the administration of an antagonist and was repeated 10 min after each dose of the blocker.

During these experiments arterial blood pressure was measured in the left carotid artery via a saline/heparin filled cannula connected to a pressure transducer (Bell & Howell) from which the heart rate was continuously monitored. All outputs were displayed on a Devices MX2 recorder.

Control experiments were undertaken which showed that four consecutive 5-HT dose-response curves, measured over a period of 180 min, did not differ significantly, validating the protocol adopted in Fig. 2.

Measurement of intestinal fluid movement in-vivo

Experiments were carried out on female Sprague-Dawley rats, 170–190 g, deprived of food for 24 h before the experiment but with free access to water. The rats were anaesthetized with sodium pentobarbitone (65 mg kg^{-1} s.c.).

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The abdomen was opened and a polythene catheter was placed in the jejunum about 5 cm distal to the flexura duodenojejunalis and fixed by ligation. A second ligature was tied approximately 20 cm distally. One h after the preparation, 2 mL Tyrode solution was instilled into the intestinal loop and the catheter withdrawn before tying off the proximal end. 5-HT creatinine sulphate (equivalent to 7×10^{-10} mol 5-HT min^{-1}) or creatinine sulphate was infused close intra-arterially into a branch of the superior mesenteric artery (0·949 mL h^{-1}) using a perfusor (Braun-Melsungen, FRG). After 30 min the loop was removed from the animal and the volume of fluid it contained determined gravimetrically. The empty loop was weighed and fluid transport was taken as the difference between the initial and final volumes in the loop and expressed as $\mu L\ g^{-1}$ wet wt/ 30 min. The effect of 5-HT was calculated by subtracting the mean basal fluid transport (with creatinine sulphate) from each value obtained in the presence of 5-HT. When present, cisapride and ketanserin were administered s.c. 15 min before filling the intestinal loop.

Expression of results

Results are expressed as mean values ± 1 s.e. of the mean of the number of observations indicated. Significance was assessed using Student's *t*-test, paired or unpaired as appropriate. Where multiple comparisons were made (Fig. 2a, b) the significance between individual means and their respective controls was assessed by analysis of variance followed by multiple comparison analysis.

Chemicals

5-Hydroxytryptamine creatinine sulphate and creatinine sulphate were obtained from Sigma Chemical Co., St. Louis, Mo. 63178, USA and from Fluka Chemische Fabrik, Buchs SG, Switzerland. Cisapride and ketanserin were gifts from Janssen Pharmaceutica, Beerse, Belgium.

Results

Effects of cisapride and ketanserin on the 5-HT-induced rise in systolic pressure

Rapid intravenous bolus injections of 5-HT elicit changes in blood pressure that can be classified into three phases, each associated with activation of a different 5-HT receptor. The first phase is a rapid, transient fall in blood pressure accompanied by a decrease in heart rate. This constitutes the Bezold-Jarisch effect and results from a reflex stimulation of the vagus following activation of 5-HT_3 receptors on sensory afferent fibres. The second phase is a brief pressor response associated with the activation of 5-HT_2 receptors before the third phase, mediated via "5-HT_1-like" receptors, showing a relatively sustained fall in blood pressure with no accompanying fall in heart rate. These effects are illustrated inset in Fig. 1. 5-HT increased blood pressure in a dose-dependent manner (Fig. 1), but this pressor response was effectively antagonized by prior administration of either cisapride ($5{\cdot}2 \times 10^{-7}$ mol kg^{-1}) or ketanserin ($7{\cdot}6 \times 10^{-8}$ mol kg^{-1}), demonstrating effective blockade of 5-HT_2 receptors at these doses. Cisapride will also interact with 5-HT_3 receptors at the doses used in this study as is evident from its ability to antagonize the Bezold-Jarisch reflex (Dunbar et al 1986).

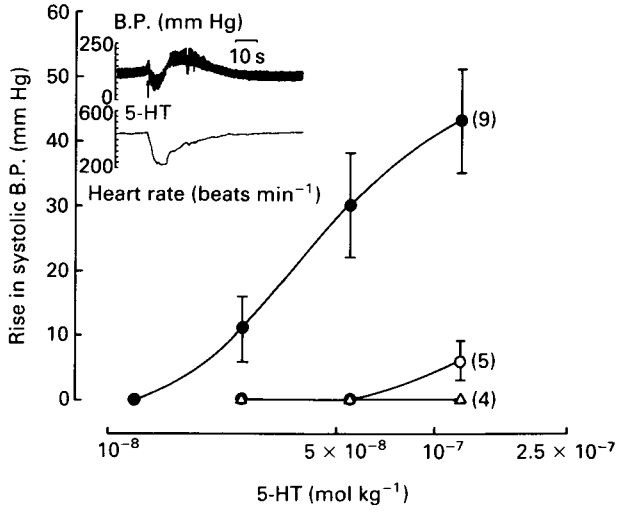

FIG. 1. Effects of cisapride and ketanserin on the rise in systolic blood pressure (B.P.) induced by 5-HT. Drugs were administered via the jugular vein and each point represents the mean ± 1 s.e. of the mean of the number of observations indicated. Inset shows the blood pressure trace with a typical response to 5-HT ($1{\cdot}65 \times 10^{-7}$ mol kg^{-1}). ● Control. ○ $5{\cdot}2 \times 10^{-7}$ mol kg^{-1} cisapride. △ $7{\cdot}6 \times 10^{-8}$ mol kg^{-1} ketansesin.

Effects of cisapride and ketanserin on the 5-HT-induced increase in the transintestinal PD

The intestinal loop generated a PD of 4–7 mV, serosa positive and this was increased transiently by 5-HT (Fig. 2). Ten min after cisapride administration the basal PD was not significantly different from its initial value. Cisapride reduced the PD changes induced by increasing doses of 5-HT and also suppressed the maximal responses (Fig. 2a), effects that were both dose-dependent. This action of cisapride does not appear to represent a non-specific inhibition of secretion as the rise in PD induced by $1{\cdot}3 \times 10^{-7}$ mol kg^{-1} PGE_2 was unaffected (control: 4·8 ± 0·4(6) mV, $+2{\cdot}1 \times 10^{-6}$ mol kg^{-1} cisapride: 4·6 ± 0·3(6) mV, $P > 0{\cdot}05$). Ketanserin had no significant effect on the basal PD nor on the changes induced by 5-HT (Fig. 2b). Both cisapride and ketanserin, at the lowest doses tested, blocked the increase in systolic blood pressure observed in response to 5-HT administration (Fig. 1).

Effects of cisapride and ketanserin on 5-HT-induced fluid secretion

Under control conditions there was a net absorption of fluid of 137 ± 24(25) $\mu L\ g^{-1}$ wet wt/30 min. 5-HT reversed this to a net secretion of 116 ± 36(22) $\mu L\ g^{-1}$ wet wt/30 min ($P < 0{\cdot}001$). Ketanserin alone has no effect on basal fluid movement (Beubler et al 1984, 1989), but in these experiments it reduced the secretory response to 5-HT in a dose-dependent manner (Fig. 3a). The failure of ketanserin to influence basal fluid movement or the 5-HT-induced changes in PD suggests that its inhibition of 5-HT-induced fluid secretion was not the result of a non-specific action. Cisapride (2.1×10^{-6} mol kg^{-1}; 8.3×10^{-6} mol kg^{-1}) had no significant effect on basal fluid movement ($P > 0.05$ in both cases). A stimulation of fluid secretion can be demonstrated using a 15 min incubation period, but the transient nature of this response is evident from the brief rise in short-circuit

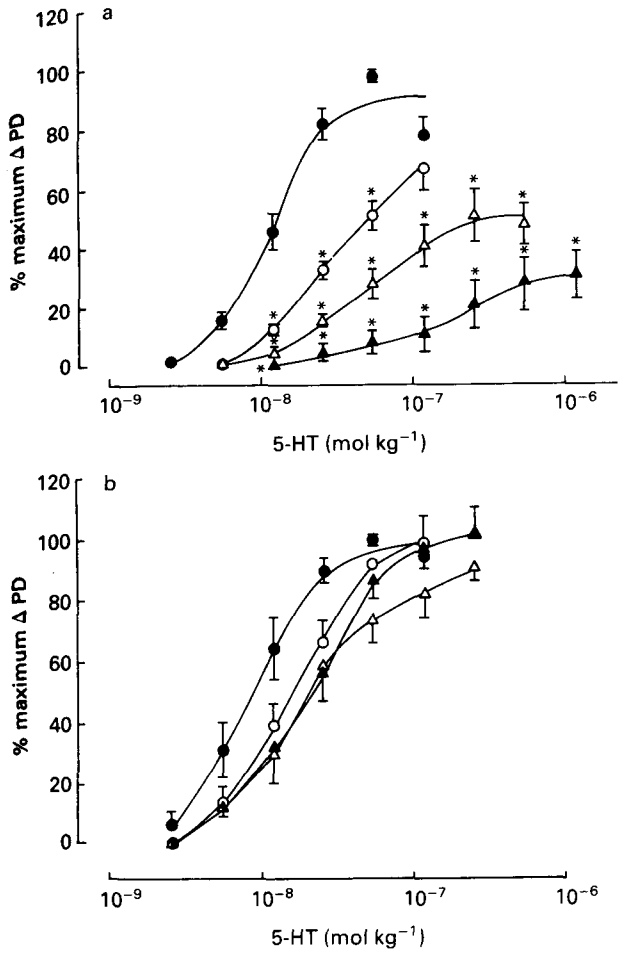

FIG. 2. Effects of cisapride (a) and ketanserin (b) on the increase in PD (ΔPD) across rat small intestine in-vivo induced by 5-HT. Drugs were administered via the jugular vein and each point represents the mean ± 1 s.e. of the mean, expressed as % control value, of the number of observations indicated. *Indicates a significant difference ($P<0.05$) from the control value. The responses to the higher doses of 5-HT applied in the presence of the antagonists were compared with the maximal control response to 5-HT. Key; a: ● control, ○ 5.2×10^{-7}, △ 2.1×10^{-6}, ▲ 8.3×10^{-6} cisapride mol kg^{-1}. n=8 100% Δ PD=4.0±0.2 (8) mV. b: ● control, ○ 7.6×10^{-8}, △ 3.0×10^{-7}, ▲ 1.2×10^{-6} ketanserin mol kg^{-1}. n=5. 100% Δ PD=42±0.3(5) mV.

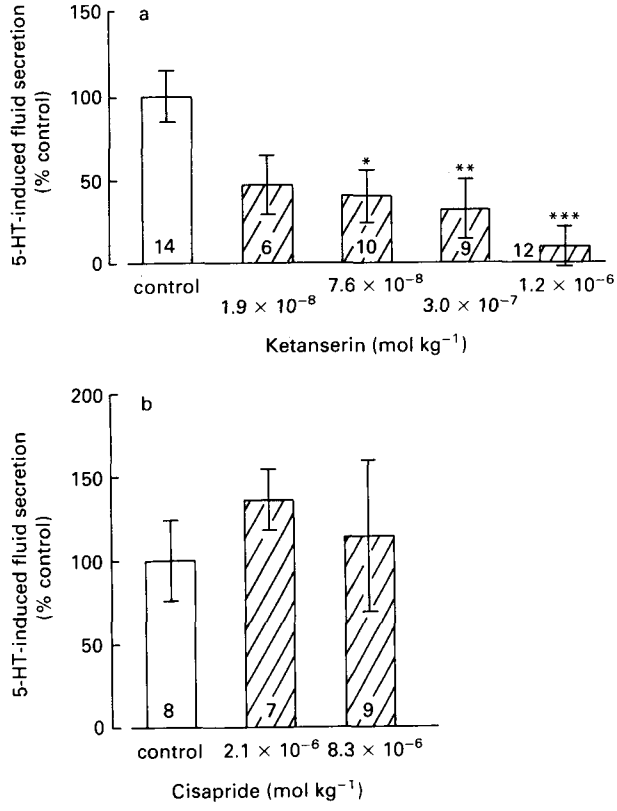

FIG. 3. Effects of ketanserin (a) and cisapride (b) on the intestinal fluid secretion in the rat induced by 5-HT (7×10^{-10} mol min^{-1} intra-arterially). Ketanserin and cisapride were administered subcutaneously 15 min before commencing the determination of fluid transport. Fluid transport is expressed as % of the control value (246±36(22) μL g^{-1} wet wt/30 min) and each bar represents the mean ± 1 s.e. of the mean of the number of observations indicated and significance was assessed using an unpaired *t*-test *$P<0.05$; **$P<0.01$; ***$P<0.001$.

current induced by cisapride (Hardcastle et al 1984), and this probably accounts for the failure to detect a significant secretion in the 30 min incubation period used in the present study. The fluid secretion induced by 5-HT (Fig. 3b) was not affected by cisapride. This lack of effect could not be attributed to restricted access of cisapride from its subcutaneous injection site since its actions in inhibiting the 5-HT-induced rises in PD were similar when cisapride was administered either subcutaneously or intravenously.

Discussion

Ketanserin and cisapride act as 5-HT receptor antagonists, and both are considered to affect 5-HT$_2$ receptors (Bradley et al 1986; Schuurkes & Van Nueten 1985). Ketanserin is considered to be particularly useful in characterizing 5-HT$_2$ receptors, being inactive at 5-HT$_3$ receptors and having little affinity for the "5-HT$_1$-like" receptor (Bradley et al 1986). Ketanserin does however, have some affinity for α-adrenoceptors but the involvement of such receptors in the actions of 5-HT reported here can be ruled out. The 5-HT-induced rise in the transintestinal PD in-vivo is unaffected by the α-antagonist phenoxybenzamine (Hardcastle et al 1981) and α-adrenoreceptor stimulation enhances fluid absorption, not fluid secretion (Levens et al 1979). Although cisapride has been used as an antagonist for 5-HT$_2$ receptors (Schuurkes & Van Nueten 1985; Moriarty et al 1987) it has been shown to exhibit weak 5-HT-M receptor antagonism as assessed using the Bezold-Jarisch reflex (Dunbar et al 1986). The classical 5-HT receptor groups of D and M receptors (Gaddum & Picarelli 1957) have been subsumed into the more recent classification of 5-HT$_2$ and 5-HT$_3$, respectively (Bradley et al 1986), although there is now doubt over whether the M receptor is indeed a 5-HT$_3$ receptor (Fozard 1987). In spite of their possible affinity for other receptors, the ability of cisapride and ketanserin to antagonize the 5-HT-induced rise in systolic blood pressure is confirmation of their affinity for 5-HT$_2$ receptors (Fig. 1). However, ketanserin and cisapride acted differently in their ability to block the actions of 5-HT on fluid transport and the transmural PD in the small intestine. Ketanserin inhibited 5-HT-induced fluid secretion without affecting the electrical responses to 5-HT (Figs 2b,

3a), while in contrast, cisapride had no effect on 5-HT-induced fluid secretion but reduced the electrical responses (Figs 2a, 3b). The ability of cisapride to antagonize the electrical response to 5-HT (measured as an increase in short-circuit current) has been noted previously in a variety of in-vitro preparations including guinea-pig stripped ileum (Cooke & Carey 1984), rat stripped ileum (Moriarty et al 1987) and rat stripped descending colon (Bunce et al 1988), as has the lack of effect of ketanserin on the electrical responses to 5-HT induced in rat stripped ileum (Ball et al 1988), guinea-pig stripped ileum and colon (Baird & Cuthbert 1987) and rat stripped descending colon (Bunce et al 1988). However, the actions of these agents on 5-HT-induced fluid secretion has not previously been reported. The present study indicates that the 5-HT receptors responsible for the effects of 5-HT on fluid secretion and electrical activity in the intestine must be of two types—one stimulating electrogenic Cl^- secretion and inhibited by cisapride but not by ketanserin, the other inhibiting electroneutral NaCl absorption and antagonized by ketanserin but not by cisapride. By definition (Bradley et al 1986), the effect on NaCl absorption must be mediated by 5-HT_2 receptors, while that on Cl^- secretion is not. However, the inability of cisapride to block 5-HT-induced fluid secretion in spite of demonstrating antagonism against 5-HT_2-mediated changes in blood pressure raises the possibility of subtypes of 5-HT_2 receptors. Another 5-HT_2 antagonist, mianserin, shares with cisapride the ability to inhibit the electrical response to 5-HT (Hardcastle et al 1981). It also reduces nutrient absorption by the small intestine, an effect that is not observed with either cisapride or ketanserin (Hardcastle et al 1986).

The net movement of fluid across the intestine is determined by the net movement of solute. The fluid secretion induced by 5-HT results from its ability to both inhibit electroneutral NaCl absorption and to stimulate electrogenic Cl^- secretion. Cisapride reduced the electrical response to 5-HT, but failed to influence 5-HT-induced fluid secretion. It would therefore appear to be capable of antagonizing the actions of 5-HT on the Cl^- secretory process without altering its effects on NaCl absorption. This is consistent with the movement of fluid being primarily linked to the cotransport of NaCl, since an inhibition of Cl^- secretion is not reflected in reduced fluid secretion. Ketanserin had contrasting actions. This agent failed to influence the electrical response of the intestine to 5-HT, but reversed the 5-HT-induced inhibition of NaCl absorption. Under these circumstances the Cl^- still secreted in response to 5-HT would be reabsorbed by the NaCl cotransport mechanism and so net ion secretion would not be enhanced, thus accounting for the inhibition of 5-HT-induced fluid secretion.

Although 5-HT influences both NaCl absorption and Cl^- secretion, the receptors responsible for these effects appear to be different. There is still no concensus regarding the location of these 5-HT receptors that regulate intestinal ion transport. Specific 5-HT binding sites have not been detected on rat intestinal epithelial cell membranes (Gaginella et al 1983), although in chicken isolated enterocytes, 5-HT has been shown to inhibit sodium absorption by releasing endogenous Ca^{2+} (Hirose & Chang 1988). Tetrodotoxin and atropine inhibit 5-HT-induced increases in the short-circuit current across guinea-pig ileum (Cooke & Carey 1985), suggesting that 5-HT does not interact with receptors on the enterocyte but indirectly via a cholinergic mechanism. In contrast, tetrodotoxin does not affect the action of 5-HT in rat colon (Zimmerman & Binder 1984), nor does atropine antagonize the 5-HT-induced rise in the PD across rat small intestine (Hardcastle et al 1981), so further clarification of the site of the 5-HT receptors is required. 5-HT influences both electrogenic and electroneutral ion transport mechanisms. Attempts to characterize the 5-HT receptors that regulate these processes solely by determining the effects of a variety of 5-HT antagonists on the electrical response to 5-HT which have concluded that the receptor type cannot be designated 5-HT_1-like, 5-HT_2 or 5-HT_3 (Bunce et al 1988), must be interpreted with caution because, even if successful, it only provides information about one aspect of 5-HT action in the regulation of intestinal ion transport.

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