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5-Hydroxytryptamine-induced Cl^- transport is mediated by 5-HT_3 and 5-HT_4 receptors in the rat distal colon

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Abstract

In the rat distal colon, 5-hydroxytryptamine (5-HT)-induced Cl^- secretion is seen as a rise in short circuit current (I_{sc}). We investigated the 5-HT receptor mediating 5-HT-induced Cl^- secretion in the rat distal colon. Rat distal colon was prepared either by stripping away the muscularis propria with the neural ganglia, or by leaving it intact. The tissue was mounted in Ussing chambers and short circuited. 5-HT receptor agonist-induced changes (Δ) in I_{sc} were recorded in the presence and absence of 5-HT receptor antagonists. In stripped preparations, the rank order of potency of agonists was: 5-HT > 5-methoxytryptamine > α-methyl-5-HT $\gg$ 2-methyl-5-HT. 5-HT and 5-methoxytryptamine-induced changes in I_{sc} were antagonized by ≥ 0.3 μM tropisetron with pA_2 values 6.5 and 6.4, respectively. The 5-HT_4 antagonist, SC 53606, antagonized the 5-HT-induced response with a pA_2 of 7.2. 5-$HT_{1\text{-like}}$ (methysergide), 5-HT_{1P} (*N*-acetyl-5-hydroxytryptophyl 5-hydroxytryptophan amide (5-HTP-DP)), 5-HT_{2A} (ketanserin) and 5-HT_3 (ondansetron) receptor antagonists had no significant effect on the 5-HT response in stripped tissue. 3 μM forskolin, or 10 μM 3-isobutyl-1-methyl-xanthine (IBMX), decreased the EC_{50} and increased the maximum 5-HT response. The 2-methyl-5-HT and 5-HT-induced ΔI_{sc} in the unstripped colon preparation were antagonized by the 5-HT_3 antagonist, ondansetron (0.3 nM), and 2-methyl-5-HT activity was abolished by pretreatment with tetrodotoxin. In conclusion, 5-HT-induced ΔI_{sc} is neurally mediated via a 5-HT_3 receptor, and non-neurally mediated via a 5-HT_4 receptor in the rat distal colon.

Keywords: Intestinal; Secretion; 5-HT receptor; Short-circuit current; cAMP

1. Introduction

5-Hydroxytryptamine (5-HT) induces electrogenic chloride ion (Cl^-) secretion that can be measured as a rise in short circuit current (I_{sc}) in the rat distal colon (Zimmerman and Binder, 1984), and in the guinea pig ileum (Cooke and Carey, 1985). The physiological and pathological functions of 5-HT have not been established. Donowitz and Binder (1975), however, have demonstrated that 5-HT can induce water and electrolyte flux in the carcinoid syndrome and may contribute to diarrhea in this condition.

In vitro studies using the rat distal colon demonstrated that 5-HT-induced Cl^- secretion is tetrodotoxin-insensitive and, therefore, likely to be non-neurally mediated (Zimmerman and Binder, 1984; Bunce et al., 1991). Conversely, in the guinea pig, 5-HT-induced Cl^- secretion has been reported to be tetrodotoxin-sensitive suggesting that it is neurally mediated (Cooke and Carey, 1985; Baird and Cuthbert, 1987). Siriwardena et al. (1991) recently reported that in the rat distal colon, Cl^- secretion is mediated by tetrodotoxin-sensitive and tetrodotoxin-insensitive pathways. Bunce et al. (1991) have also recently explored the role of a tetrodotoxin-insensitive pathway for 5-HT-induced Cl^- secretion in the guinea pig ileum. Bunce found that the tetrodotoxin-insensitive pathway may be mediated by a 5-$HT_{4\text{-like}}$ receptor.

The 5-$HT_{1\text{-like}}$ receptor subtypes have so far not been demonstrated to have any activity in electrolyte transport (Bunce et al., 1991). The role of the 5-HT_{2A} receptor is unclear but may be responsible for inhibition of neutral NaCl transport in the rat (Beubler et al., 1990). This receptor subtype has been isolated on crypt-enriched enterocytes from the guinea-pig ileum (Siriwardena et al., 1993b). The 5-HT_3 receptor has been demonstrated to mediate electrogenic Cl^- secretion in both rat (Siriwardena et al., 1993a) and guinea pig (Baird and Cuthbert,

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1987) via a tetrodotoxin-sensitive pathway. The more recently described 5-HT_4 receptor has been found to have a wide distribution peripherally. There has, therefore, been interest in a putative role of the 5-HT_4 receptor in Cl^- secretion. In this respect, recent reports provide evidence suggesting that the 5-HT_4 receptor may mediate the tetrodotoxin-resistant secretion of Cl^- in the rat distal colon (Bunce et al., 1991) and in the guinea pig ileum (Baird and Cuthbert, 1987).

In the present study, the aim was to examine neural and non-neural 5-HT receptor pathways that mediate 5-HT-induced ΔI_{sc} in the rat distal colon. Further, we aimed to confirm and consolidate findings from this and other laboratories.

2. Materials and methods

Sprague Dawley rats (average weight 400 g) were allowed free access to standard rat chow and water. They were killed by carbon dioxide-induced narcosis followed by decapitation. The distal colon was excised and prepared either by stripping away the muscularis propria and neural ganglia, or using the full thickness of the colon, unstripped. These were cut into flat sheets and mounted in Ussing chambers (window area 0.75 cm^2). Tissue was bathed in oxygenated (95% O_2-5% CO_2) Krebs solution (115 mM NaCl, 25 mM $NaHCO_3$, 24 mM K_2HPO_4, 0.4 mM KH_2PO_4, 1.2 mM $CaCl_2.2H_2O$, and 1.2 mM $MgCl_2 \cdot 6H_2O$, pH 7.4) on both sides and maintained at 37°C. Ascorbic acid (1 mM) was added to the bathing solutions to prevent oxidation of 5-HT. Dextrose (10 mM) was added to both sides of the chambers as a nutrient source. The chambers were connected to voltage clamps (DVC-1000; WPI, New Haven, CT, USA) and subjected to short circuit conditions for the duration of the experiment with the exception of brief interruptions for reading the potential difference. A 30 min equilibration period was allowed before addition of pharmacological agents.

2.1. Concentration-response of agonists in the presence or absence of antagonists

5-HT agonist concentration-response curves were constructed by a cumulative method using one chamber for the generation of each concentration-response curve. As reported by Bunce et al. (1991), multiple curves cannot be constructed by using one chamber, since repeated washing of the tissue causes diminished responses. This phenomenon was also observed in our laboratory. Four to six chambers were used simultaneously with adjacent pieces of colon in the chambers receiving different treatments or acting as controls. After the maximum response at each concentration (usually within 2–3 min), a higher concentration of agonist was added. For antagonist studies, the tissue was pretreated with an antagonist for 20 min prior to the addition of an agonist. The change in short circuit current (ΔI_{sc}) was recorded as the response to adding the agonist.

2.2. Data analysis

Equipotent concentration ratios were calculated graphically from the EC_{50} response level. All EC_{50} values were calculated for individual concentration-response curves using the actual change in I_{sc} to plot the response by logistic curve fitting on the statistical computer program, SAS (SAS Institute, Cary, NC, USA.). From these plots, the mean EC_{50} values were used to make a Schild plot and estimate the pA_2 values as described by Arunlakshana and Schild (1959). The pK_B values were calculated using the equation:

$$pK_b = \mathrm{Log}\left(\frac{EC_{50}\ \text{agonist with antagonist}}{EC_{50}\ \text{agonist}} - 1\right) - \mathrm{Log}\,(\text{antagonist concentration})$$

Statistics were analyzed by 1-way ANOVA (analysis of variance) Tukey post test ($P \leq 0.05$ significant). The concentration-response curves shown in the results are the percentages of the means of n experiments and expressed as percentages of the maximum 5-HT-induced ΔI_{sc}.

2.3. Drugs

The drugs used were: 5-HT creatinine sulphate, tetrodotoxin, 5-methoxytryptamine, 3-isobutyl-1-methylxanthine (IBMX), forskolin and ketanserin (obtained from Sigma Chemicals, St.Louis, MO, USA). 2-Methyl-5-hydroxytryptamine and α-methyl-5-hydroxytryptamine (from RBI, Natick, MA, USA). Methysergide (Sandoz Pharmaceuticals, Basel, Switzerland), ondansetron (Glaxo pharmaceuticals, London, UK), tropisetron (a gift from Sandoz). (1-*S*,8-*S*)-*N*-[(hexahydro-1*H*-pyrrolizin-1-yl)methyl]-6-chloroimidazo[1,2-*a*]pyridine-8-carboxamide hydrochloride (SC 53606) was kindly donated by Searle Pharmaceuticals (Stokie, IL, USA) and *N*-acetyl-5-hydroxytryptophyl 5-hydroxytryptophan amide (5-HTP-DP) was purchased from New York Psychiatric Institute (New York, NY, USA). All compounds were dissolved in distilled water with the exception of IBMX and forskolin which had to be dissolved in ethanol and then diluted in distilled water.

3. Results

In the stripped preparation 5-HT (0.01–100 μM) induced a concentration-dependent ΔI_{sc}. The baseline I_{sc} values before and after treatment with tetrodotoxin (0.2 μM) were compared by an analysis of covariance to the controls and found to be significantly different; tetrodotoxin lowers the I_{sc}. Tetrodotoxin pretreatment did not signifi-

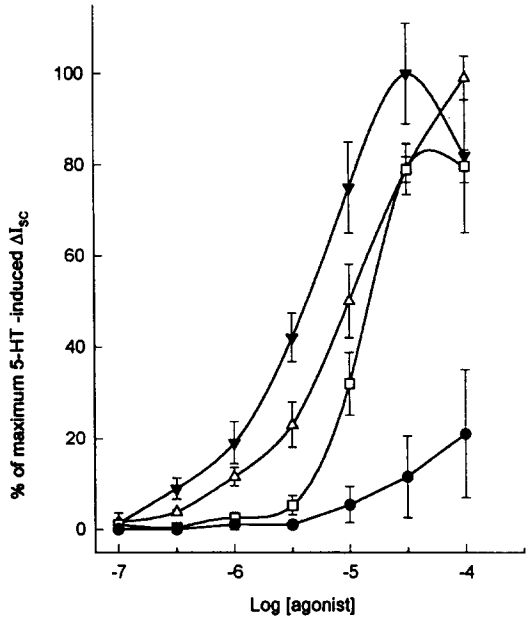


Fig. 1. The change in short circuit current (I_{sc}) in the stripped rat distal colon in response to: 5-hydroxytryptamine (5-HT), 5-methoxytryptamine, α-methyl-5-HT and 2-methyl-5-HT in the stripped colon: (▼) 5-HT, (△) 5-methoxytryptamine, (□) α-methyl-5HT, (●) 2-methyl-5-HT. Values are the means ± S.E.M ($n = 5$).

cantly change the response to 5-HT; the EC_{50} with tetrodotoxin was 5.1 ± 0.7 μM compared to 5.8 ± 0.6 μM in the control group ($n = 5$). The effect of tetrodotoxin in the stripped preparation is suggestive of some retained neural elements. To abolish neural conduction effects and obviate our concerns about inconsistency in the stripping process, tetrodotoxin pretreatment was done routinely in this preparation.

To determine whether any significant active uptake or breakdown of 5-HT occurs during the experiments, some ($n = 3$) were performed in the presence and absence of 1 mM pargyline (monoamine oxidase inhibitor) and 1 μM fluoxetine (a neural 5-HT uptake inhibitor) in unstripped rat distal colon. There were no changes in the 5-HT concentration-response curve (data not shown) and these compounds were, therefore, not routinely used in the experiments.

3.1. Responses in the stripped tetrodotoxin-treated distal colon

5-HT (EC_{50} 4.8 ± 0.8 μM, $n = 15$), 5-methoxytryptamine (EC_{50} 12.0 ± 1.8 μM, $n = 5$) and α-methyl-5-HT (EC_{50} 16.5 ± 1.9 μM, $n = 5$) induced concentration dependent (0.1–100 μM) changes in I_{sc}. 2-Methyl-5-HT (0.1–100 μM), however, produced only a very weak concentration-dependent change in I_{sc} (Fig. 1). 5-Methoxytryptamine induced a maximum response which was 99% of the 5-HT-induced change in I_{sc}, and α-methyl-5-HT produced a maximum of 80% of the 5-HT-induced maximum change in I_{sc}. This suggests that α-methyl-5-HT may be a partial agonist. This limits the comparison of agonist activity of α-methyl-5-HT to 5-HT. 2-Methyl-5-HT, at 100 μM, induced only a 14% increase in the 5-HT-induced maximum change in I_{sc}. With the caveat of the partial agonist activity of α-methyl-5-HT, the

Table 1

5-HT receptor	Antagonist ($n = 5$–6)	EC_{50}	
		5-HT	5-HT + antagonist
5-HT_{1P}	5-HTP-DP (10 μM)	4.2 ± 0.8	4.2 ± 0.8
$5\text{-HT}_{1\text{-like}}$, 5-HT_2	Methysergide (1.0 μM)	5.9 ± 1.0	5.7 ± 0.5
5-HT_{2A}	Ketanserin (0.1 μM)	5.0 ± 0.5	5.2 ± 1.0
5-HT_3	Ondansetron (0.3 μM)	5.8 ± 0.4	6.1 ± 0.5

The EC_{50} values for ΔI_{sc} in response to 5-HT in the stripped rat distal colon; in the presence and absence of the 5-HT receptor antagonists which had no significant effect on EC_{50} for 5-HT.

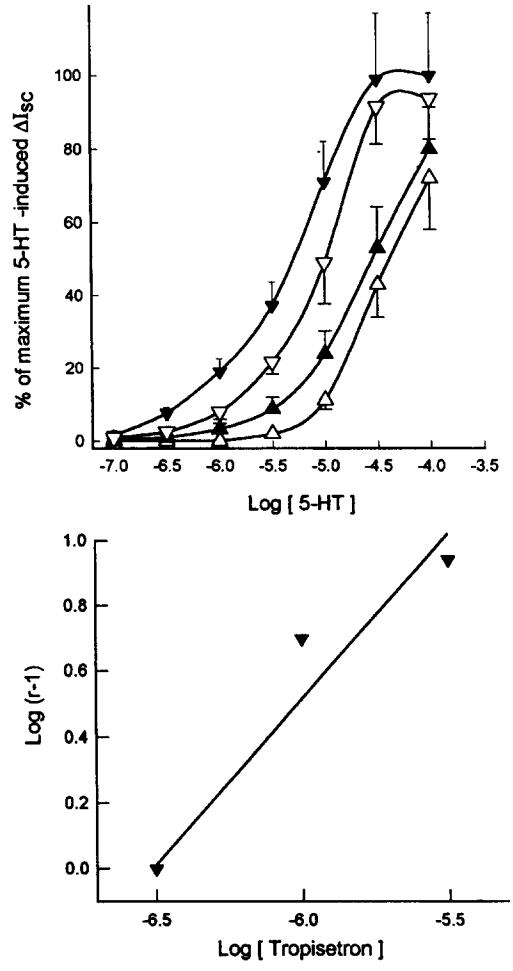
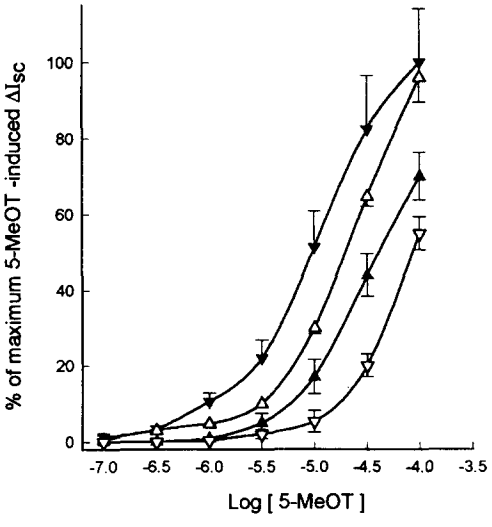


Fig. 2. Top: the effect of tropisetron on the 5-hydroxytryptamine (5-HT)-induced change in short circuit current (I_{sc}) in isolated stripped rat distal colon: (▼) 5-HT control; (▽) tropisetron 0.3 μM; (▲) tropisetron 1 μM; (△) tropisetron 3 μM. Values are the means ± S.E.M. ($n = 5$). Bottom: the Schild plot using tropisetron. The slope was 0.9, and when constrained to unity, the pA_2 was 6.5.

rank order of agonist potency ratio is: 5-HT > 5-methoxytryptamine > α-methyl-5-HT ≫ 2-methyl-5-HT.

Pretreatment of the tissue ($n = 5$) with 1 μM methysergide (5-$HT_{1\text{-like}}$ and 5-HT_2 receptor antagonist), 10 μM 5-HTP-DP (5-HT_{1P} receptor antagonist), 0.1 μM ketanserin (5-HT_{2A} receptor antagonist), or 0.3 μM ondansetron (5-HT_3 receptor antagonist) had no significant effect on the EC_{50} for 5-HT (Table 1). The maximum 5-HT-induced response was unaffected by the presence of any of these antagonists. None of the 5-HT receptor antagonists significantly affected the baseline I_{sc} per se in either tissue preparation.

Tropisetron at 0.3, 1 and 3 μM produced dextral shifts in the 5-HT (Fig. 2, top) and 5-methoxytryptamine (Fig. 3, top) concentration-response curves. The shifts in EC_{50} were significant at all concentrations of tropisetron used



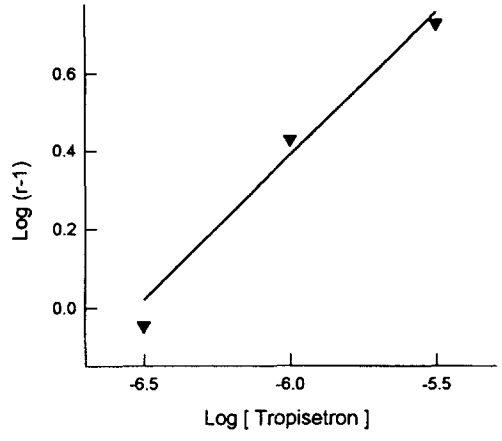
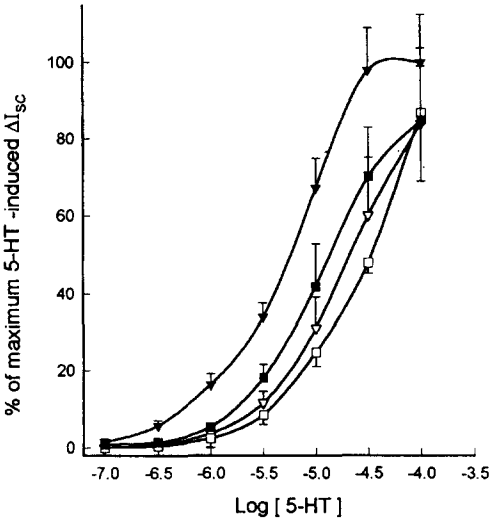


Fig. 3. Top: the effect of tropisetron on the 5-methoxytryptamine-induced change in short circuit current (I_{sc}) in isolated stripped rat colon: (▼) 5-methoxytryptamine control; (△) tropisetron 0.3 μM; (▲) tropisetron 1 μM; (▽) tropisetron 3 μM. Values are the means ± S.E.M. ($n = 5$). Bottom: the Schild plot for tropisetron and using 5-methoxytryptamine as the agonist. The slope was 0.8, and when constrained to unity, the pA_2 was 6.4.



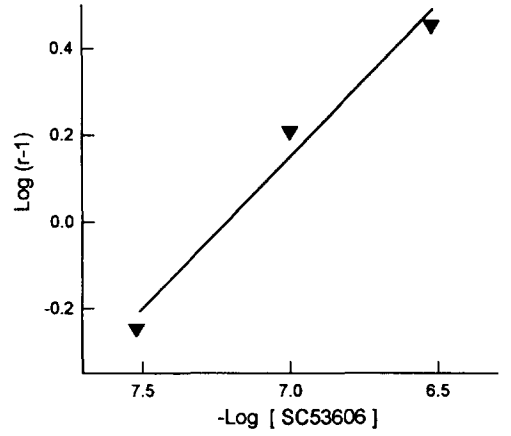


Fig. 4. Top: the effect of SC 53606 on the 5-hydroxytryptamine (5-HT)-induced rise in short circuit current (I_{sc}) in isolated stripped rat colon: (▼) 5-HT control; (■) SC53606 0.03 μM; (▽) SC53606 0.1 μM; (□) SC53606 0.3 μM. Values are the means ± S.E.M. ($n = 5$–6). Bottom: the Schild plot for SC 53606. The slope was 0.7, and when constrained to unity, the pA_2 was 7.2.

with both 5-HT and 5-methoxytryptamine. The Schild plot slopes were 0.9 for 5-HT (Fig. 2, bottom) and 0.8 for 5-methoxytryptamine (Fig. 3, bottom). These slopes were not significantly different from unity. When constrained to unity, the pA_2 values were 6.5 and 6.4, respectively.

The new, selective 5-HT_4 receptor antagonist, SC 53606, produced dextral shifts in the 5-HT concentration-response curve when used at concentrations of 0.03, 0.1 and 0.3 μM (Fig. 4, top). The shift in EC_{50} was significant at 0.1 and 0.3 μM. The Schild plot had a slope of 0.7 which was not significantly different from unity. When constrained to unity, the pA_2 was 7.2 (Fig. 4, bottom).

Pretreating the tissue with 3 μM forskolin produced a significant rise in the baseline I_{sc}. When 5-HT was added, the maximum induced change in I_{sc} was significantly increased when compared to 5-HT-induced ΔI_{sc} in the absence of forskolin (Fig. 5). The concentration-response

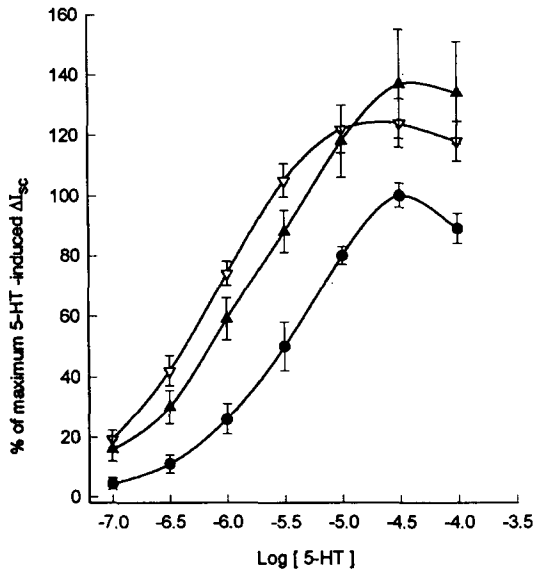


Fig. 5. The change in short circuit current (I_{sc}) in the stripped rat distal colon in response to 5-hydroxytryptamine (5-HT) alone (●), or 5-HT in the presence of 10 μM IBMX (▲) or 3 μM forskolin (▽). The maximum responses are expressed as percentages of the response 5-HT induces alone. Values are the means ± S.E.M. (n = 3–5).

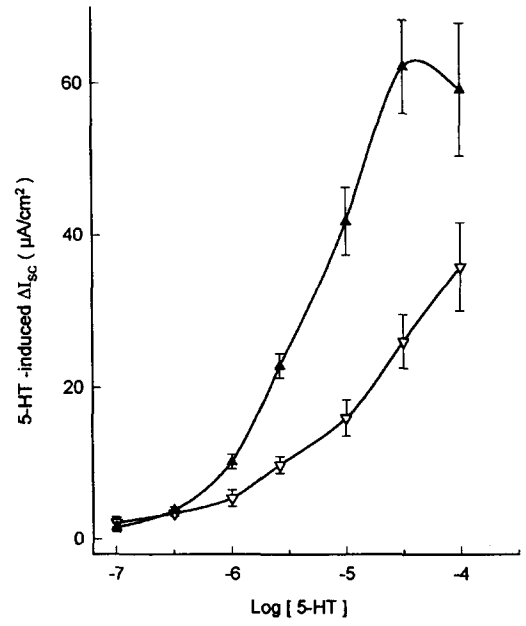


Fig. 6. The 5-HT-induced rise in short circuit current (I_{sc}) in the stripped (▲) and unstripped (▽) rat distal colon. Values are the means ± S.E.M. (n = 5).

curve was also shifted to the left and the EC_{50} was decreased from 3.1 ± 0.3 μM to 1.3 ± 0.6 μM (n = 5).

Pretreating the tissue with 10 μM IBMX caused a brief rise in I_{sc} after which a new stable I_{sc} was assumed, and this was significantly elevated over the pretreatment value (covariance analysis, n = 5). IBMX pretreatment also caused a significant increase in sensitivity to 5-HT. The concentration-response curve was moved to the left: the EC_{50} decreasing from 3.5 ± 0.8 μM to 0.8 ± 0.1 μM (n = 5). The maximum ΔI_{sc} was also significantly (P < 0.05, ANOVA) elevated to 130% of the 5-HT-induced control value (Fig. 5).

3.2. Response in the unstripped tissue

The 5-HT-induced change in I_{sc} in the unstripped tissue was lower than the change seen in the stripped tissue. Higher concentrations of 5-HT were required to produce the same response as in the stripped tissue (Fig. 6). The EC_{50} in the stripped preparation was 5 μM. To produce the same response in the unstripped tissue required a 5-fold increase in 5-HT; even at 100 μM, 5-HT still did not produce the same maximal response.

In the unstripped preparation both 5-HT and 2-methyl-5-HT (0.1–100 μM) produced a concentration-dependent change in I_{sc} (Fig. 7). At 100 μM, 2-methyl-5-HT produced 70% of the 5-HT-produced change in I_{sc}. The 2-methyl-5-HT-produced change in I_{sc} was abolished by the presence of tetrodotoxin (Fig. 7). When the tissue was pretreated with 3 nM ondansetron (5-HT_3 receptor antagonist), the EC_{50} for 2-methyl-5-HT increased from 3.9 ± 0.7 μM to 8.4 ± 0.7 μM with a pK_B of 8.5. The maximum 2-methyl-5-HT response was not significantly affected by ondansetron (Fig. 7).

In the unstripped preparation treatment with 5-HTP-DP (10 μM), methysergide (1 μM) and ketanserin (0.1 μM) had no effect on the 5-HT-induced ΔI_{sc} (data not shown). The ΔI_{sc} produced by 30 μM 5-HT, decreased significantly (P < 0.05, ANOVA) from 33.0 ± 8.0 μA/cm^2 to

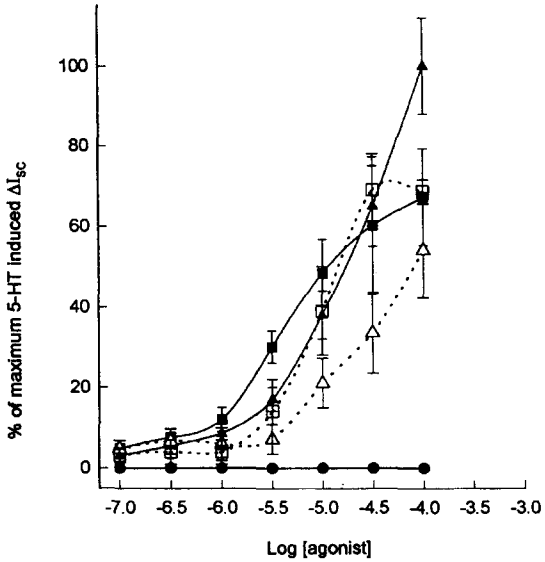


Fig. 7. The change in short circuit current (I_{sc}) response to 5-HT (▲), 2-methyl-5-HT (■), 5-HT in the presence of 3 nM ondansetron (△), 2-methyl-5-HT in the presence of 3 nM ondansetron (□) or 2-methyl-5-HT in the presence of 0.2 μM tetrodotoxin (●) in unstripped tissue. Values are the means ± S.E.M. (n = 5).

14.3 ± 2.5 $\mu A/cm^2$ in the presence of 3 nM ondansetron ($n = 4$) (Fig. 7).

4. Discussion

We have demonstrated that the 5-HT-induced change in I_{sc} is mediated via a neural and non-neural pathway. A 5-HT_3 receptor mediates the neural pathway, and our study indicates that the non-neural pathway is mediated by a 5-HT_4 receptor. The non-neural pathway was isolated using rat distal colon stripped of neural ganglia and pretreated with tetrodotoxin. A neural pathway was also isolated using unstripped rat distal colon. To examine the non-neural pathways one must effectively inhibit the neural pathways. This cannot be guaranteed by the stripping process alone. Tetrodotoxin was, therefore, added to block nerve conduction in any remnants of nerve tissue. Tetrodotoxin itself has been shown to have no effect on the transport properties of mucosal sheets (Biagi et al., 1990). In our experiment the presence of tetrodotoxin did not affect the 5-HT-induced ΔI_{sc}, demonstrating that in the absence of neural conduction there remains a concentration-dependent ΔI_{sc} in response to 5-HT. A non-neural, tetrodotoxin-insensitive pathway for 5-HT-induced ΔI_{sc} was previously demonstrated by Zimmerman and Binder (1984) in the rat distal colon. Conversely, Cooke and Carey (1985) and later Baird and Cuthbert (1987) reported that in the guinea pig, the 5-HT-induced rise in I_{sc} was tetrodotoxin-sensitive. Scott et al. (1992) recently illustrated the presence of a tetrodotoxin-insensitive pathway in the guinea-pig ileum. This pathway may be mediated by the 5-HT_4 receptor. However, the evidence provided by his study was not conclusive and suggested that there may be other 5-HT receptor subtypes involved in the mediation of changes in short circuit current. Our results support the findings of Cooke et al. (1991) that the 5-HT_3 receptor mediates the neural pathway, and further, indicates that the 5-HT_4 receptor mediates the non-neural pathway.

The response to 5-HT in the Ussing chamber model is a ΔI_{sc}. Since this has been amply demonstrated to be due to net Cl^- ion secretion both by electrolyte flux studies (Zimmerman and Binder, 1984) and by pharmacological methods (Bouhelal et al., 1988), the ΔI_{sc} was accepted as a measure of Cl^- secretion in this study.

The $5\text{-HT}_{1\text{-like}}$ and 5-HT_2 receptor antagonist, methysergide, the 5-HT_{1P} receptor antagonist, 5-HTP-DP, and the 5-HT_{2A} receptor antagonist, ketanserin, had no effect on the 5-HT-induced ΔI_{sc} either in the stripped or unstripped preparation. Beubler et al. (1990) suggest that the 5-HT_2 receptor may inhibit neutral NaCl absorption without demonstrable electrical changes. The inhibition of neutral sodium chloride flux does not induce a change in I_{sc}, and therefore cannot be assessed by this method.

5-HT, 5-methoxytryptamine and α-methyl-5-HT all produced an agonist response in the stripped rat distal colon. 2-Methyl-5-HT, conversely, had very weak agonist potency. 5-Methoxytryptamine was approximately equipotent to 5-HT, having a similar EC_{50} and producing a similar maximum response. α-Methyl-5-HT was less potent than 5-methoxytryptamine and behaved like a partial agonist. This finding is similar to that published by Scott et al. (1992), who found that α-methyl-5-HT was a partial agonist in the guinea pig ileum. In comparing these potencies, it must be noted that α-methyl-5-HT does not act as a full agonist. 2-Methyl-5-HT, had very little agonist activity in the stripped colon. The rank order of agonist potency in this study was: 5-HT > 5-methoxytryptamine > α-methyl-5-HT $\gg$ 2-methyl-5-HT. This agonist profile is typical of the 5-HT_4 receptor (Craig and Clarke, 1990) and similar to the activity reported by Scott et al. (1992) in the guinea pig ileal mucosa. 2-Methyl-5-HT has little agonist potency at the 5-HT_4 receptor. In contrast, it is a relatively potent agonist (as compared to 5-HT) at the 5-HT_3 receptor (Richardson et al., 1985).

The effects of both 5-HT and 5-methoxytryptamine were antagonized by concentrations of ≥ 0.3 μM tropisetron with pA_2 values of 6.4 and 6.5 respectively. Tropisetron has a relatively high affinity for the 5-HT_3 receptor (pA_2 8–10) (Richardson et al., 1985) compared to its affinity for the 5-HT_4 receptor (pA_2 6–6.5) (Craig and Clarke, 1990). The pA_2 in this study is within the reported range for the 5-HT_4 receptor. Further, the absence of neural conduction in this preparation makes activity of tropisetron at a 5-HT_3 receptor unlikely since the 5-HT_3 receptor has only been found on neural tissue (Richardson and Engel, 1986). In this study, neural ganglia were removed by stripping and any remaining nerve conduction blocked by tetrodotoxin.

Recently, more specific 5-HT_4 receptor antagonists have become available. We found that one of these, SC 53606, antagonized the 5-HT-induced rise in I_{sc}. SC 53606 has been reported to have a pA_2 of 7.8 at the 5-HT_4 receptor and to have very little affinity for the 5-HT_3 receptor (Yang et al., 1993). We found in our study that SC 53606 antagonized the 5-HT-induced rise in I_{sc} and from the Schild plot calculated the value of pA_2 as 7.2. This is lower than the reported pA_2 (Yang et al., 1993) value but compatible with activity at a 5-HT_4 receptor. In our study the slope of the Schild plot was 0.7. This, however, was not significantly different from a slope of unity. At present, there are no comparable studies of this antagonist in a similar model. Although there should be little variation of the pA_2 value due to tissue variation, differences in pA_2 values have sometimes been observed (Leff and Martin, 1989). It is noteworthy that the pA_2 observed in the rat distal colon is consistent with a value of 7.3 that we observed in the human jejunal mucosal membrane (unpublished observation).

It has been reported that 5-HT_4 receptor activation stimulates cyclic AMP production (Dumuis et al., 1988). It would, therefore, be anticipated that agents affecting cyclic

AMP production and breakdown will affect the response produced by 5-HT at a 5-HT_4 receptor. Pretreatment with forskolin, a non-specific cyclic AMP stimulator, has been shown to have synergistic effects on hormones which act via cyclic AMP. We found that 3 μM forskolin increased the sensitivity of the rat colon to 5-HT, thereby causing both a rise in the maximum I_{sc} response as well as a decrease in the EC_{50}.

The phosphodiesterase inhibitor, IBMX, reduces the rate of cyclic AMP breakdown and, therefore, is expected to act synergistically with 5-HT. We found that pretreatment with IBMX significantly increased the sensitivity of the tissue to 5-HT, as previously described in the guinea pig (Scott et al., 1992). In contrast to this report, however, we also demonstrated an increase in the 5-HT-induced maximal change in I_{sc} with IBMX pretreatment. This can be explained by the increased accumulation of cyclic AMP in the 5-HT-stimulated cell, pretreated with IBMX.

The response of the unstripped preparation is different to that of the stripped preparation. The stripped preparation appears to be more sensitive to 5-HT. Kenakin (1984) has illustrated the difficulties in comparing agonist efficacy in different tissue preparations. The response to 2-methyl-5-HT, a preferential 5-HT_3 receptor agonist, in the stripped and unstripped preparation is different. In the stripped, tetrodotoxin-pretreated preparation which is more sensitive to 5-HT, 2-methyl-5-HT at 100 μM induced only 14% of the 5-HT-induced change in I_{sc} but in the unstripped tissue 100 μM 2-methyl-5-HT induced 70% of the 5-HT-induced change in I_{sc}. 2-Methyl-5-HT appeared to be a more potent agonist in the unstripped preparation. Its action in the unstripped preparation was antagonized by tetrodotoxin. In the unstripped preparation 2-methyl-5-HT appears to be acting preferentially at the 5-HT_3 receptor. This is confirmed by the activity of the selective 5-HT_3 receptor antagonist ondansetron with a pK_B of 8.5. The 5-HT_3 receptor is effectively removed in the stripped, tetrodotoxin-treated tissue. The weak activity of 2-methyl-5-HT in the stripped, tetrodotoxin-treated preparation is likely to be at the 5-HT_4 receptor, whose activity is unmasked by the absence of the 5-HT_3 receptor. Another possibility is that the stripping of the tissue removes a barrier to the diffusion of 2-methyl-5-HT to the vicinity of the 5-HT_4 receptor. 2-Methyl-5-HT has, however, only very little agonist efficacy at the 5-HT_4 receptor (Craig et al., 1990). This pathway is similar to the tetrodotoxin-sensitive pathway described by Cooke and Carey (1985) in the guinea pig ileum. The 5-$HT_{1\text{-like}}$ and 5-HT_2 receptor antagonist, methysergide, the 5-HT_{1P} receptor antagonist, 5-HTP-DP, and the 5-HT_{2A} receptor antagonist, ketanserin, did not influence the tissue response to 5-HT in the unstripped preparation.

In summary, we have demonstrated that in the rat distal colon, there is a tetrodotoxin-sensitive and tetrodotoxin-insensitive pathway mediating Cl^- secretion. The tetrodotoxin-sensitive pathway is mediated via a 5-HT_3 receptor and the tetrodotoxin-insensitive pathway is mediated via a 5-HT receptor which has characteristics making it identifiable as a 5-HT_4 receptor.

References

Arunlakshana, O. and H.O. Schild, 1959, Some quantitative uses of drug antagonists, Br. J. Pharmacol. 14, 48.

Baird, A.W. and A.W. Cuthbert, 1987, Neuronal involvement in type 1 hypersensitivity reactions in gut epithelia, Br. J. Pharmacol. 92, 647.

Beubler, E., I.M. Coupar, J. Hardcastle and P.T. Hardcastle, 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.

Biagi, B., Y.Z. Wang and H.J. Cooke, 1990, Effects of tetrodotoxin on chloride secretion in rabbit distal colon: tissue and cellular studies, Am. J. Physiol. 258, G223.

Bouhelal, R., L. Smounya and J. Bockaert, 1988, 5-HT_{1B} receptors are negatively coupled with adenylate cyclase in rat substantia nigra, Eur. J. Pharmacol. 151, 189.

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

Cooke, H.J. and H.V. Carey, 1985, Pharmacological analysis of 5-hydroxytryptamine actions on guinea-pig ileal mucosa, Eur. J. Pharmacol. 111, 329.

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

Craig, D.A. and D.E. Clarke, 1990, Pharmacological characterization of a neuronal receptor for 5-hydroxytryptamine in guinea pig ileum with properties similar to the 5-hydroxytryptamine receptor, J. Pharmacol. Exp. Ther. 252, 1378.

Craig, D.A., R.M. Eglen, L.K. Walsh, L.A. Perkins, R.L. Whiting and D.E. Clarke, 1990, 5-Methoxytryptamine and 2-methyl-5-hydroxytryptamine-induced desensitization as a discriminative tool for the 5-HT_3 and putative 5-HT_4 receptors in guinea pig ileum, Naunyn-Schmied. Arch. Pharmacol. 342, 9.

Donowitz, M.D. and H.J. Binder, 1975, Jejunal fluid and electrolyte secretion in carcinoid syndrome, Dig. Dis. 20(12), 1115.

Dumuis, A., R. Bouhelal, M. Sebben, R. Cory and J. Bockaert, 1988, A nonclassical 5-hydroxytryptamine receptor positively coupled with adenylate cyclase in the central nervous system, Mol. Pharmacol. 34, 880.

Kenakin, T.P., 1984, The classification of drugs and drug receptors in isolated tissues, Pharmacol. Rev. 36(3), 165.

Leff, P. and Martin, G.R., 1989, Quantification of the actions of 5-Hydroxytryptaminereceptor agonists and antagonists, in: The Peripheral Actions of 5-Hydroxytryptamine, ed. J.R. Fozard (Oxford University Press) p. 26.

Richardson, B.P. and G. Engel, 1986, The pharmacology and function of 5-HT3 receptors, Trends Pharmacol. Sci. 8, 424.

Richardson, B.P., G. Engel, P. Donatsch and P.A. Stadler, 1985, Identification of serotonin M-receptor subtypes and their specific blockade by a new class of drugs, Nature 316, 126.

Scott, C.M., K.T. Bunce and C.F. Spraggs, 1992, Investigation of the 5-hydroxytryptamine receptor mediating the maintained short-circuit current response in guinea-pig ileal mucosa, Br. J. Pharmacol. 106, 877.

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

Siriwardena, A.K., M.R. Budhoo, E.P. Smith and J.M. Kellum, 1993a, A

5-HT_3 receptor agonist induces neurally mediated chloride transport in rat distal colon, J. Surg. Res. 55, 55.

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

Yang, D., B. Goldstin, A.E. Moormann, D.L. Flynn and G.W. Gullickson, 1993, SC 53606, A potent and selective antagonist of 5-hydroxytryptamine$_4$ receptors in isolated rat oesophageal muscularis mucosae, J. Pharmacol. Exp. Ther. 266(3), 1339.

Zimmerman, T.W. and H.J. Binder, 1984, Serotonin-induced alteration of colonic electrolyte transport in the rat, Gastroenterology 86, 310.