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The role of the 5-HT_4 receptor in Cl^- secretion in human jejunal mucosa

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Abstract

5-Hydroxytryptamine (5-HT) is a mediator of chloride ion (Cl^-) secretion in the intestine which can be seen as a rise in short circuit current (I_{sc}) in the Ussing chamber model. We investigated the 5-HT receptor mediating 5-HT-induced Cl^- secretion in the human jejunum in vitro. Jejunal segments obtained from patients having gastric bypass surgery for obesity, were stripped of muscularis and mounted in Ussing chambers and short-circuited. The 5-HT receptor agonist-induced change (Δ) in I_{sc} was recorded in the presence and absence of 5-HT receptor antagonists. The rank order of agonist potency was: 5-HT > 5-methoxytryptamine > renzapride (BRL 24924) > α-methyl-5-HT > > 2-methyl-5-HT. In the presence of Cl^--free media or 100 μM furosemide, 5-HT-induced ΔI_{sc} was significantly reduced. It was also antagonized by $\geq$ 1 μM tropisetron (a 5-HT_3/5-HT_4 receptor antagonist) and $\geq$ 10 nM GR 113808 (a selective 5-HT_4 receptor antagonist) with pA_2 values of 6.5 and 7.9, respectively. Another 5-HT_4 receptor antagonist, SC 53606 (0.1 μM), antagonized the 5-HT-induced response with a pA_2 of 7.3. 5-HT_1-like/5-HT_2 (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 and tetrodotoxin, had no significant effect on the EC_{50} for 5-HT. In conclusion, this study demonstrates that in the human muscle-stripped jejunum in vitro, 5-HT induced change in short circuit current is mediated by a 5-HT_4 receptor via a non-neural pathway.

Keywords: Cl^-; Cl^- secretion; 5-HT receptor; Short circuit current; Jejunum; (human)

1. Introduction

Serotonin (5-HT, 5-hydroxytryptamine) is known to be a potent secretagogue for intestinal chloride ions (Cl^-) and water (Donowitz and Binder, 1975) and a mediator of diarrhea in the carcinoid syndrome (Kowlessar, 1989). Much recent interest has focused upon the mechanism by which 5-HT induces Cl^- secretion. Tetrodotoxin-sensitive (Baird and Cuthbert, 1987; Siriwardena et al., 1991) and tetrodotoxin-insensitive pathways (Zimmerman and Binder, 1984) have been described in animal studies. The presence of a tetrodotoxin-insensitive pathway leaves open the possibility that the involved 5-HT receptor could be located on the mucosal cell surface. Gaginella et al. (1983), however, were unable to find a 5-HT binding site on mucosal enterocytes from rat colon. More recently, Siriwardena et al. (1993b) have demonstrated 5-HT_2 receptors on enterocytes isolated in suspension from the guinea pig ileum. Scott et al. (1992), however, suggested that there may be more than one receptor subtype mediating 5-HT-induced Cl^- secretion via a tetrodotoxin-insensitive pathway in the guinea pig ileum. Whilst a great deal of progress has been made in defining the pathway of 5-HT-induced Cl^- secretion, the receptor(s) involved are not fully characterized.

Very little is known about how 5-HT acts in the human intestine. We recently published evidence of Cl^- secretory activity induced by exogenous 5-HT in the human jejunum in vitro (Kellum et al., 1994). Radioisotopic flux studies documented that the change in short-circuit current could be accounted for by net electrogenic Cl^- secretion. This report indicated that the receptor appears to be the 5-HT_4 receptor type, although no highly selective 5-HT_4 receptor antagonists were available at that time to facilitate confirmation. It has been reported that selective antagonism of 5-HT_4 receptors in muscle-strippped preparations of human terminal ileum reduces 5-HT-induced secretion (Burleigh and Borman, 1993, Borman and Burleigh, 1993). The present study aims to consolidate previous findings and to further characterize the 5-HT receptor(s) that mediate Cl^- secretion in the human jejunum in vitro.

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2. Materials and methods

2.1. Materials and methods

Segments of jejunum were obtained from patients having gastric bypass surgery for obesity. Informed consent was given in each case for use of the tissue. The jejunum was opened at the mesenteric border and the muscle layers were removed by sharp dissection. The tissue was mounted in Ussing chambers with window aperture diameter of 1.13 cm^2 and was oxygenated with 95% O_2/5% CO_2 whilst being maintained at 37°C. To the Krebs solution on both sides of the tissue 10 mM dextrose and 10 mM ascorbic acid were routinely added. Dextrose provided a nutrient source, and ascorbic acid was added to reduce oxidation of 5-HT. The tissue was short circuited by a voltage clamp (DVC-1000; WPI, New Haven, CT, USA).

Electrical measurements of potential difference (PD) and short circuit current (I_{sc}) were made. The conductance (G), the reciprocal of the resistance, was calculated from (I_{sc}/PD = G). Krebs agar bridges placed within 1 mm of the membrane surface were connected to calomel electrodes by a solution of saturated KCl. The electrodes were connected to the potentiometer terminals of a voltage clamp. The external direct current was applied across the membrane via two AgCl electrodes. These electrodes were in NaCl solution and connected to the ends of the chambers by agar bridges as described. The electrodes were connected to the variable electromotive force terminals of the same voltage clamps. The tissue was short-circuited for the duration of the experiments except for when reading the potential difference.

2.2. Pharmacological studies

Using cumulative additions, 5-HT receptor agonist concentration-response curves were made using 0.1–100 μM solutions of: 5-HT, 5-methoxytryptamine, renzapride (BRL 24924), α-methyl-5-HT and 2-methyl-5-HT. The change in I_{sc} was recorded. The 5-HT concentration-response data were analyzed in the presence and absence of the following receptor antagonists: 10 μM *N*-acetyl-5-hydroxytryptophyl 5-hydroxytryptophan amide (5-HTP-DP, a 5-HT_{1P} receptor antagonist), 1 μM methysergide (a 5-HT_1-like and 5-HT_2 receptor antagonist), 0.1 μM ketanserin (a 5-HT_{2A} receptor antagonist), 0.3 μM ondansetron (a 5-HT_3 antagonist), 0.3, 1 and 3 μM tropisetron (a selective 5-HT_3 receptor antagonist at $\leq$ 0.05 μM and a 5-HT_4 receptor antagonist at $\geq$ 0.1 μM), 3, 10 and 30 nM GR 113808, a recently developed selective 5-HT_4 receptor antagonist, and 0.1 μM SC 53606, another recently characterized 5-HT_4 receptor antagonist. None of the 5-HT receptor antagonists significantly affected the equilibrium I_{sc} alone. The effect of furosemide (100 μM) pretreatment and Cl^--free media were also investigated.

2.3. Calculations

The pA_2 values were calculated from Schild plots (Arunlakshana and Schild, 1959) or by the method of MacKay (1978), using the Schild equation:

pA_2 = log [(EC_{50} agonist in presence of antagonist)/(EC_{50} agonist) − 1] − log (antagonist concentration)

The concentration-response curves were analyzed individually by logistic curve fitting on the computer program SAS (SAS Insitute, Cary, NC, USA). The EC_{50} for each paired receptor agonist and antagonist study was calculated from these curves and then mean EC_{50} calculated. These EC_{50} values were used to plot a Schild plot. Statistical analysis of the EC_{50} values was done by 1-way ANOVA (analysis of variance) tukey post test ($P \leq 0.05$ significant).

The slope of the Schild plot was computed by linear regression (Pharmacological Calculation Systems, Version 4.2, 1991 (Developed by RJ Tallirida and RB Murray, Philadelphia, PA, USA)). If the slope was not significantly different from unity (95% confidence limit) the pA_2 was calculated from the slope.

2.4. Drugs and solutions

Krebs solution in this study was composed of: 115 mM NaCl, 25 mM $NaHCO_3$, 24 mM K_2HPO_4, 0.4 mM KH_2PO_4, 1.2 mM $CaCl_2 \cdot 2H_2O$ and 1.2 mM $MgCl_2 \cdot 6H_2O$. Dextrose (10 mM) and ascorbic acid (1 mM) was added.

The drugs used were: 5-HT creatinine sulphate, tetrodotoxin, furosemide, 5-methoxytryptamine and ketanserin (obtained from Sigma Chemicals, St Louis, MO, USA). 2-Methyl-5-hydroxytryptamine, α-methyl-5-hydroxyryptamine (from RBI, Natick, MA, USA). Methysergide (Sandoz Pharmaceuticals, Basel, Switzerland), ondansetron and GR 113808 (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). Renzapride (BRL 24924) was kindly donated by Smith, Kline and Beecham Pharmaceuticals (Betchworth, Surrey, UK) and *N*-acetyl-5-hydroxytryptophyl 5-hydroxytryptophan amide (5-HTP-DP) was purchased from the New York Psychiatric Institute (New York, NY, USA). All compounds were dissolved in distilled water with the exception of tropisetron which had to be dissolved in ethanol and then made up to strength in distilled water.

3. Results

3.1. Effect of furosemide and Cl^--free media on 5-HT receptor agonist response

The 5-HT concentration response curve was significantly depressed in the presence of furosemide or a Cl^--free

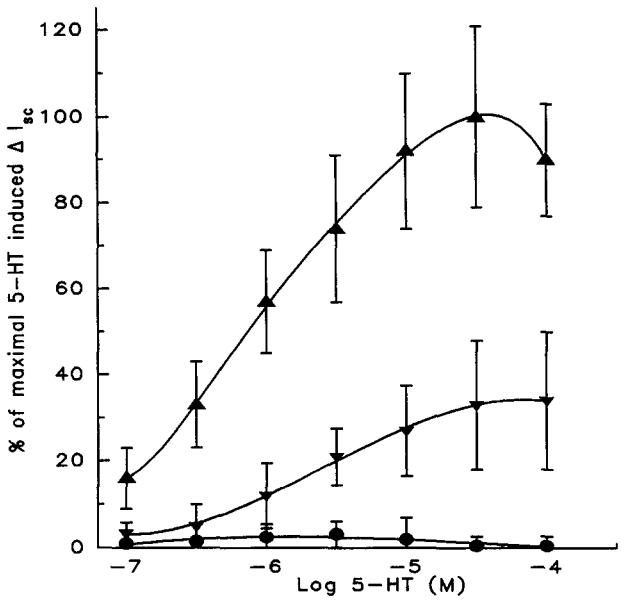

Fig. 1. 5-HT concentration-response curve in the presence of 100 μM of furosemide (▼) or in Cl^--free media (•). When compared to the control curve (▲), there was significant depression of the maximum response induced by 5-HT at all concentrations of 5-HT ($n = 5$). ANOVA, $P \leq 0.05$ significant.

media (Fig. 1). The response was more marked in the Cl^--free media. In this part of the study ascorbic acid was replaced by pargyline since ascorbic acid oxidizes furosemide. Cl^--free Krebs solution was made by substituting NaCl with Na isoethionate. The very small contribution of Cl^- from the Mg^{2+} and Ca^{2+} salts were ignored since they represented less than 1% of the Cl^- content of the Krebs solution and, therefore, were unlikely to significantly affect the total Cl^- content of the medium.

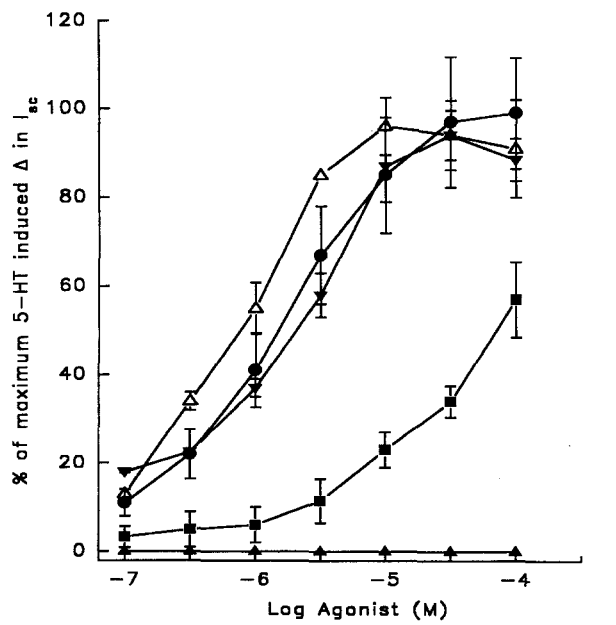

Fig. 2. Agonist concentration-response curves using 0.1–100 μM of: 5-HT (△), renzapride (•), 5-methoxytryptamine (▼), α-methyl-5-HT (■) and 2-methyl-5-HT (▲) ($n = 5–8$).

Table 1
The effect of agonists on the short circuit current response in the human jejunum

Agonist	Equipotent molar ratio	Percentage of 5-HT maximum
5-HT	1	100 ± 14
5-Methoxytryptamine	1.3	94 ± 12
Renzapride	0.8	96 ± 7
α-Methyl-5-HT	42 [a]	57 ± 9 [a]
2-Methyl-5-HT	No significant effect up to 100 μM	

$n = 5–8$. [a] Values are based on a sub-maximal agonist response within the studied concentration range.

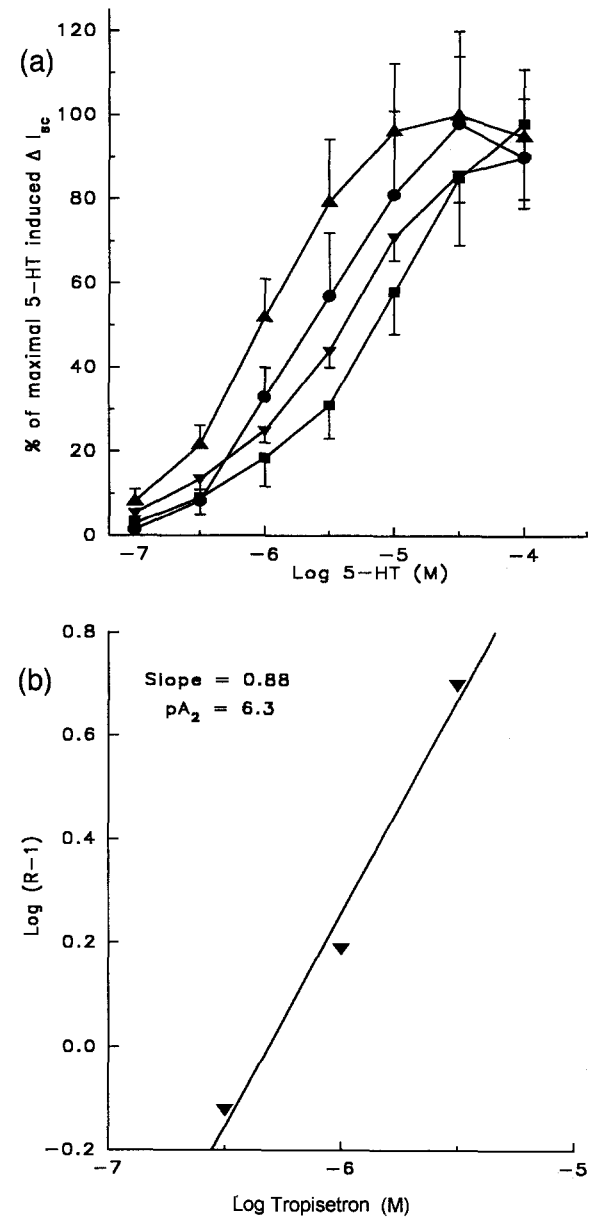

Fig. 3. (a) 5-HT concentration-response curve in the presence of tropisetron at 0.1 (•), 1 (▼), and 3 (■) μM and compared to the 5-HT control (▲). There is a progressive shift in the 5-HT concentration-response curve in the presence of increasing concentrations of tropisetron ($n = 4–6$). (b) Schild plot analysis of 5-HT concentration-response curve in the presence of tropisetron. The slope is not significantly different from unity and the pA_2 value is 6.3.

3.2. Characterization by use of specific 5-HT receptor agonists

The following 5-HT receptor agonists were used in concentrations of 0.1–100 μM, serially increasing the concentrations: 5-HT, 5-methoxytryptamine, renzapride, α-methyl-5-HT and 2-methyl-5-HT. 5-HT induced a concentration-dependent change in I_{sc}. The maximum response to 5-HT was observed at 30 μM with the EC_{50} at 1 ± 0.1 μM. In the same manner, 5-methoxytryptamine, renzapride and α-methyl-5-HT produced a rise in I_{sc} (Fig. 2). The maximum response to α-methyl-5-HT was not achieved in this concentration range. 2-Methyl-5-HT did not have any observed effect on the baseline I_{sc}.

The ratio of the potency of the agonists were compared (Table 1). 5-HT was the most potent followed by 5-methoxytryptamine and renzapride (approximately equipotent). α-Methyl-5-HT had little and 2-methyl-5-HT had no agonist potency. The potency ratio shows that 5-HT > 5-methoxytryptamine > renzapride > α-methyl-5-HT > > 2-methyl-5-HT.

3.3. Pharmacological characterization of the 5-HT response in the presence of antagonists

A 5-HT concentration-response curve was produced in the presence and absence of tropisetron. This antagonist was used at 0.3, 1 and 3 μM. There was a dextral shift in the concentration response curve (Fig. 3a). This shift was increased in the presence of increasing concentrations of tropisetron. The antagonism was surmountable and the increase in the EC_{50} for 5-HT (Table 2) was significant at 1 and 3 μM tropisetron. The slope of the Schild plot for tropisetron was 0.88. This was not significantly different from a slope of unity, and the calculated pA_2 was 6.3 (Fig. 3b).

Table 2
Summary of results of antagonists which resulted in a shift in the EC_{50} for 5-HT, and the concentration ratio of EC_{50} for 5-HT in the presence and absence of different concentrations of antagonists

Antagonist ($n = 4$–8)	EC_{50}	
	5-HT + antagonist	5-HT
Tropisetron		
0.3 μM	3.2 ± 0.9	1.8 ± 0.6
1.0 μM	3.0 ± 0.2 [a]	1.2 ± 0.1
3 μM	5.4 ± 1.6 [a]	0.9 ± 0.1
GR 113808		
0.003 μM	1.6 ± 0.1	1.2 ± 0.1
0.01 μM	2.4 ± 0.3 [a]	1.3 ± 0.1
0.03 μM	4.0 ± 0.3 [a]	1.2 ± 0.1
SC 53606		
0.1 μM	5.7 ± 1.0	1.7 ± 0.5

Values are the means ± S.E.M in μM. [a] Significant (ANOVA, $P \leq 0.05$) compared to control EC_{50} for 5-HT.

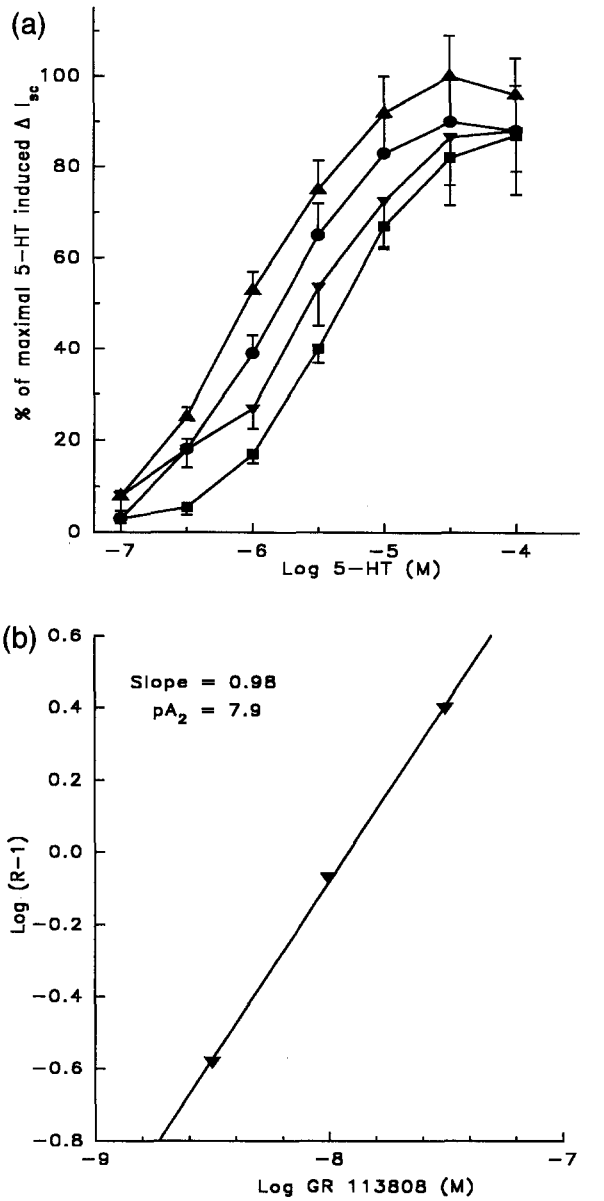

Fig. 4. (a) 5-HT concentration-response curve in the presence of GR 113808 at 3 (·), 10 (▼), and 30 (■) nM and compared to the 5-HT control (▲). There is a progressive shift in the 5-HT concentration-response curve in the presence of increasing concentrations of GR 113808 ($n = 4$–8). (b) Schild plot analysis of 5-HT concentration-response curve in the presence of GR 113808. The slope is not significantly different from unity and the pA_2 value is 7.9.

The new, selective 5-HT_4 receptor antagonist, GR 113808, was employed at 3, 10 and 30 nM. In the presence of GR 113808, there was a shift in the 5-HT concentration response curve to the right (Fig. 4a). This shift progressed dextrally with increasing concentrations of GR 113808. The increase in EC_{50} for 5-HT was significant in the presence of 10 and 30 nM GR 113808 (Table 2). The slope of the Schild plot (Fig. 4b) for GR 113808 was 0.98 which was not significantly different from unity, and the pA_2 was 7.9.

SC 530606 caused a dextral shift in the 5-HT concentra-

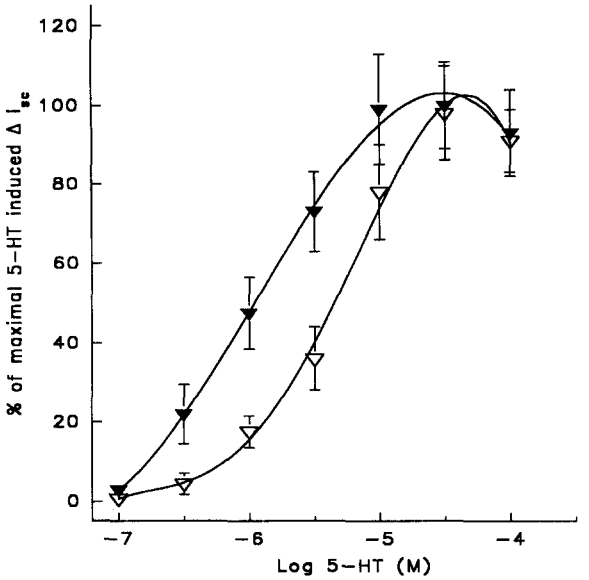

Fig. 5. 5-HT concentration-response curve in the presence (▽) and absence (▼) of 0.1 μM of SC 53606 ($n = 5$).

tion response curve (Fig. 5). The shift in the region of 20–80% was found to approximate a parallel shift in the 5-HT concentration-response curve to the right. The EC_{50} was significantly increased (Table 1) and the antagonism was surmountable. The calculated pK_B was 7.3.

Methysergide (1 μM), 5-HTP-DP (10 μM), ketanserin (0.1 μM) and ondansetron (0.3 μM) had no significant effect on the 5-HT concentration-response curve or the EC_{50} for 5-HT. Tetrodotoxin (0.1 μM), an inhibitor of neural conduction, also failed to significantly affect the EC_{50} for 5-HT (Table 3).

4. Discussion

The results of this study are consistent with the presence of a 5-HT_4 receptor-mediated, tetrodotoxin-insensitive response to 5-HT in muscle-stripped human jejunum in the Ussing chamber model. We have previously demonstrated, using radioisotopic techniques in flux chambers, that the 5-HT-induced ΔI_{sc} in the human jejunum is due to net Cl^- secretion (Kellum et al., 1994). A similar Cl^- secretory mechanism for the ΔI_{sc} induced by 5-HT has been demonstrated by pharmacological means in the guinea pig (Cooke and Carey, 1985). The present study reaffirms this mechanism in chambered human intestine by pharmacological means. Treatment with furosemide significantly inhibited and Cl^--free medium abolished 5-HT-induced ΔI_{sc}. It has, therefore, been amply demonstrated that 5-HT causes net Cl^- secretion, which can be monitored as a ΔI_{sc}, in human jejunum.

Table 3
Summary of results with antagonists that had no significant effect (by ANOVA) on the EC_{50} for 5-HT

Antagonist ($n = 4$–6)	Receptor	EC_{50}	
		5-HT	5-HT + antagonist
5-HTP-DP (10 μM)	5-HT_{1P}	1.5 ± 0.2	1.3 ± 0.3
Methysergide (1 μM)	5-$HT_{1\text{-like}}$, 5-HT_2	1.1 ± 0.1	1.8 ± 0.3
Ketanserin (0.1 μM)	5-HT_2	2.1 ± 0.4	2.4 ± 0.6
Ondansetron (0.3 μM)	5-HT_3	1.6 ± 0.1	1.6 ± 0.3
Tetrodotoxin (0.2 μM)	Neural conduction inhibitor	1.4 ± 0.4	1.8 ± 0.6

Values are the means ± S.E.M. in μM.

It is known that the transport effects of 5-HT are mediated by neuronal and non-neuronal pathways in the chambered rat distal colon (Budhoo and Kellum, 1994; Siriwardena et al., 1993a). The 5-HT_3 receptor appears to be the primary receptor mediating the neuronal pathway in a non-muscle-stripped version of this model (Budhoo and Kellum, 1994). The I_{sc} response to 5-HT was 5-HT_3-receptor antagonist-sensitive and abolished by tetodotoxin. The present study in muscle-stripped human jejunal mucosa has demonstrated the 5-HT-induced ΔI_{sc} to be insensitive to both tetrodotoxin and a 5-HT_3 receptor antagonist. These same characteristics have been observed in a second, distinct pathway in the rat distal colon (Budhoo and Kellum, 1994).

Amongst the 5-HT receptor antagonists examined in this study, only tropisetron and the more selective 5-HT_4 receptor antagonists, GR113808 and SC53606, had any effect on the 5-HT-induced ΔI_{sc}. The 5-HT_{1P} receptor antagonist, 5-HTP-DP, had no significant effect even at a high concentration. This receptor has been characterized within the intestinal neural plexus (Mawe et al., 1986), which was removed by the stripping process in this study. The 5-HT_1-like and 5-HT_2 receptor antagonist, methysergide, the 5-HT_{2A} receptor antagonist, ketanserin, and the selective 5-HT_3 receptor antagonist, ondansetron, were all ineffective antagonists in the present study.

Tropisetron antagonized the 5-HT-induced change in I_{sc} with a pA_2 value consistent with that of a 5-HT_4 receptor (Clarke et al., 1989). Tropisetron acts preferentially at the 5-HT_3 receptor, where it has a high affinity (Richardson et al., 1985). It attenuated the response to 5-HT only at concentrations of ≥ 0.3 μM, whilst only significantly altering the EC_{50} at ≥ 1 μM. This finding is consistent with antagonism at a 5-HT_4 receptor and is supported by the absence of efficacy of the more selective 5-HT_3 receptor antagonist, ondansetron. Similarly, the 5-HT_3 receptor agonist, 2-methyl-5-HT, did not induce a rise in I_{sc} in this preparation. Agonist efficacy of 5-methoxytryptamine in the absence of 2-methyl-5-HT efficacy has been demonstrated to be a means to distinguish the 5-HT_4 from the 5-HT_3 receptor types (Craig et al., 1990). The rank order of agonist potency demonstrated in this study is characteristic of the 5-HT_4 receptor (Craig and Clarke, 1990).

GR113808 has been reported to be a selective 5-HT_4 receptor antagonist with negligible activity at the other

5-HT subtypes (Grossman et al., 1993). In this study GR113808 antagonized the 5-HT-induced response in a concentration-dependent manner with a pA_2 of 7.9. This value is lower than the expected pA_2 for the 5-HT_4 receptor of 9.5; however, some variability in these values has been recognized for other receptor antagonists (Martin and Leff, 1986). Likewise, the calculated pA_2 value with SC53606 was lower than that reported for a 5-HT_4 receptor by Yang et al. (1993). A pA_2 value for this human tissue has not been reported previously but is similar to our finding in the rat distal colon using the same techniques (Budhoo and Kellum, 1994).

In conclusion, therefore, we have demonstrated that the tetrodotoxin-insensitive pathway for 5-HT-induced Cl^- secretion in the human jejunum in vitro is mediated by a 5-HT_4 receptor. The stripping technique utilized in this model, which removes most of the submucosal plexus, did not permit an examination of possible neurally mediated transport effects of serotonin.

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