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European Journal of Pharmacology 320 (1997) 187–192

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Effect of ramosetron on short-circuit current response in rat colonic mucosa

Tetsuo Kiso *, Hiroyuki Ito, Keiji Miyata

Neuroscience Research, Pharmacology Laboratories, Institute for Drug Discovery Research, Yamanouchi Pharmaceutical Co., Ltd., 21 Miyukigaoka, Tsukuba, Ibaraki 305, Japan

Received 26 August 1996; revised 1 November 1996; accepted 8 November 1996

Abstract

We investigated the effects of ramosetron (YM060, (−)-(*R*)-5-[(1-methyl-1*H*-indol-3-yl)carbonyl]-4,5,6,7-tetrahydro-1*H*-benzimidazole monohydrochloride) on the short-circuit current (I_{sc}) responses to 5-HT receptor agonists in the rat distal colon, and compared its potency to that of other 5-HT_3 receptor antagonists. 5-Hydroxytryptamine (5-HT) concentration-dependently increased I_{sc}. The I_{sc} response to 5-HT was partially reduced by tetrodotoxin and ramosetron, and strongly inhibited by GR113808 ([[1-[(2-methylsulphonyl)amino]ethyl]-4-piperidin-yl]methyl 1-methyl-1*H*-indole-3-carboxylate). 2-Methyl-5-HT and 5-methoxytryptamine also increased I_{sc}. The former response was inhibited by ramosetron, and the latter was abolished by GR113808. Ramosetron, YM114 (KAE-393, (−)-(*R*)-5-[(1-indolinyl)carbonyl]-4,5,6,7-tetrahydro-1*H*-benzimidazole monohydrochloride) and granisetron concentration-dependently antagonized the I_{sc} responses to 2-methyl-5-HT with reduction in the maximal response at higher concentrations. Apparent pA_2 values for these antagonists were 10.40, 10.37 and 8.99, respectively. Ondansetron produced clear rightward shifts of the concentration-response curves to 2-methyl-5-HT, with a pA_2 value of 8.53. These results suggest that 5-HT increases I_{sc} through the 5-HT_3 and 5-HT_4 receptors, and that ramosetron is a potent and selective 5-HT_3 receptor antagonist in rat colonic mucosa.

Keywords: 5-HT_3 receptor; Colon, distal; Short-circuit current (I_{sc}); Ramosetron (YM060, (−)-(*R*)-5-[(1-methyl-1*H*-indol-3-yl)carbonyl]-4,5,6,7-tetrahydro-1*H*-benzimidazole monohydrochloride); (Rat)

1. Introduction

Serotonin (5-hydroxytryptamine, 5-HT) is an important neurotransmitter which is implicated in a variety of physiological responses. 5-HT is known to evoke various gastrointestinal dysfunctions such as emesis, motility disorders and diarrhea (King and Sanger, 1989). It has recently been reported that gastrointestinal disorders associated with stress are, at least in part, attributable to 5-HT released from 5-HT-containing neurons and enterochromaffin cells, and that the effects of 5-HT are mediated via 5-HT_3 receptors (Miyata et al., 1992).

5-HT has a stimulative effect on the secretion of water and electrolytes in jejunum, ileum and colon in many species (Burleigh and Borman, 1993; Hansen, 1994; Johnson et al., 1994; Kellum et al., 1994). Receptor subtypes involved in this 5-HT-induced secretion are the 5-HT_2, 5-HT_3 and 5-HT_4 subtypes (Hansen and Jaffe, 1994; Hansen et al., 1994; Hardcastle and Hardcastle, 1995; Scott et al., 1992). The increase in the secretion of Cl^- in rat colonic mucosa results in an increase in short-circuit current (I_{sc}). Budhoo et al. (1996) reported that the increase in I_{sc} induced by 5-HT is mediated by 5-HT_3 and 5-HT_4 receptors in the rat distal colon.

Ramosetron (YM060, (−)-(*R*)-5-[(1-methyl-1*H*-indol-3-yl)carbonyl]-4,5,6,7-tetrahydro-1*H*-benzimidazole monohydrochloride) is a novel and selective 5-HT_3 receptor antagonist. This antagonist strongly blocks contraction of guinea pig isolated colon evoked by 5-HT and 2-methyl-5-HT (Miyata et al., 1991), suppresses 5-HT-induced defecation and diarrhea in rats, and inhibits stress-induced defecation in rats (Miyata et al., 1992). However, the effects of ramosetron on gastrointestinal secretion are not well known.

In the present study, we examined the effects of ramosetron on the I_{sc} responses to 5-HT receptor agonists

* Corresponding author. Tel.: (81-298) 52-5111, ext. 2871; Fax: (81-298) 56-2515.

PII S0014-2999(96)00893-X

in the rat distal colon, and compared its potency to that of other 5-HT_3 receptor antagonists.

2. Materials and methods

2.1. Preparations

Male Wistar rats weighing 280–350 g were used. They were killed by cervical dislocation and exsanguinated. The distal colon was removed and two adjacent preparations of the mucosa were prepared by dissection of the muscle layers (Bunce et al., 1991). These were then mounted in Ussing chambers (window area 0.8 cm^2), bathed on both sides with Krebs-bicarbonate solution warmed to 37°C and equilibrated with 95% O_2/5% CO_2. The composition of the Krebs solution was (in mM) NaCl 118.2, KCl 4.6, $MgSO_4$ 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 24.8, KH_2PO_4 1.2 and glucose 10.0. Preparations were short-circuited by use of a short-circuit current amplifier (Nihon Kohden CEZ-9100; Tokyo, Japan) and the I_{sc} was continuously recorded.

All experiments were performed in compliance with the regulations of the Animal Ethical Committee of Yamanouchi Pharmaceutical.

2.2. Experimental protocol

All concentration-response curves of agonists were constructed in a cumulative manner. For agonist studies, one preparation received 5-HT and the paired mucosa received different 5-HT receptor agonists. For antagonist studies, one preparation received agonist alone and the paired mucosa was exposed to the antagonist for 30 min before the addition of the agonist.

2.3. Statistical analysis

EC_{50} values were calculated by curve fitting using the statistical computer program SAS. The pA_2 value was determined according to the following Schild equation:

$$pA_2 = \log(\text{dose ratio} - 1) - \log(\text{antagonist concentration})$$

Apparent pA_2 values were estimated with the Schild equation at the lowest concentration of antagonist which did not reduce the maximal response to 2-methyl-5-HT.

2.4. Drugs

Ramosetron HCl, YM114 (KAE-393, (−)-(*R*)-5-[(1-indolinyl)carbonyl]-4,5,6,7-tetrahydro-1*H*-benzimidazole monohydrochloride), granisetron HCl, ondansetron HCl, GR113808 ([[1-[(2-methylsulphonyl)amino]ethyl]-4-piperidin-yl]methyl 1-methyl-1*H*-indole-3-carboxylate), 2-methyl-5-HT and *m*-chlorophenylbiguanide were prepared by Yamanouchi Pharmaceutical (Tsukuba, Japan). 5-HT creatinine sulfate was purchased from E. Merck (Darmstadt, Germany). 5-Methoxytryptamine HCl was obtained from Fluka (Buchs, Switzerland). Tetrodotoxin and hexamethonium chloride were from Sigma (St. Louis, MO, USA). Atropine sulfate was purchased from Wako (Osaka, Japan). All drugs were dissolved in Krebs solution.

3. Results

3.1. Effect of 5-HT receptor agonists and antagonists

5-HT (10^{-7}–3×10^{-4} M), 5-methoxytryptamine (3×10^{-7}–3×10^{-4} M), 2-methyl-5-HT (3×10^{-7}–3×10^{-4} M) and *m*-chlorophenylbiguanide (10^{-7}–10^{-5} M) concentration-dependently increased I_{sc} in rat colonic mucosa (Fig. 1). The EC_{50} values for 5-HT, 5-methoxytryptamine, 2-methyl-5-HT and *m*-chlorophenylbiguanide were 7.3 $(6.3–8.5)\times10^{-6}$, 1.2 $(1.0–1.5)\times10^{-5}$, 2.2 $(1.8–2.6)\times10^{-6}$ and 3.0 $(2.5–3.6)\times10^{-7}$ M, respectively. The maximal responses to 5-methoxytryptamine, 2-methyl-5-HT and *m*-chlorophenylbiguanide were 80.8 ± 8.8%, 36.4 ± 3.5% and 33.3 ± 3.3% of that to 5-HT, respectively.

The I_{sc} response to 5-HT was partially reduced by the neuronal blocker tetrodotoxin (10^{-6} M) and the 5-HT_3 receptor antagonist ramosetron (10^{-7} M), and inhibited by the 5-HT_4 receptor antagonist GR113808 (3×10^{-7} M) (Fig. 2). The response to 5-methoxytryptamine was not affected by tetrodotoxin and ramosetron, but was completely abolished by GR113808 (Fig. 3). 2-Methyl-5-HT-induced response was blocked by tetrodotoxin and ramosetron, but was not affected by GR113808 (Fig. 4).

3.2. Effect of 5-HT_3 receptor antagonists on responses to 2-methyl-5-HT

The antagonistic activities of the 5-HT_3 receptor antagonists ramosetron, YM114, granisetron and ondansetron

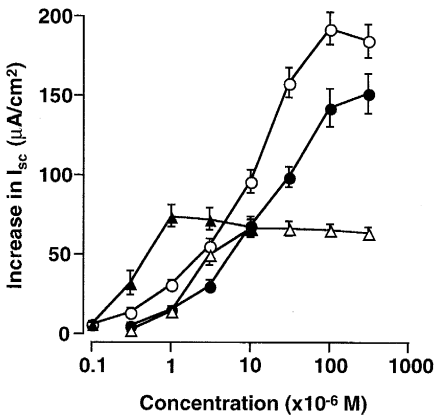

Fig. 1. The short-circuit current (I_{sc}) responses to 5-HT (○), 5-methoxytryptamine (●), 2-methyl-5-HT (△) and *m*-chlorophenylbiguanide (▲) in rat colonic mucosa. The results are the mean ± S.E.M. of 6–9 preparations.

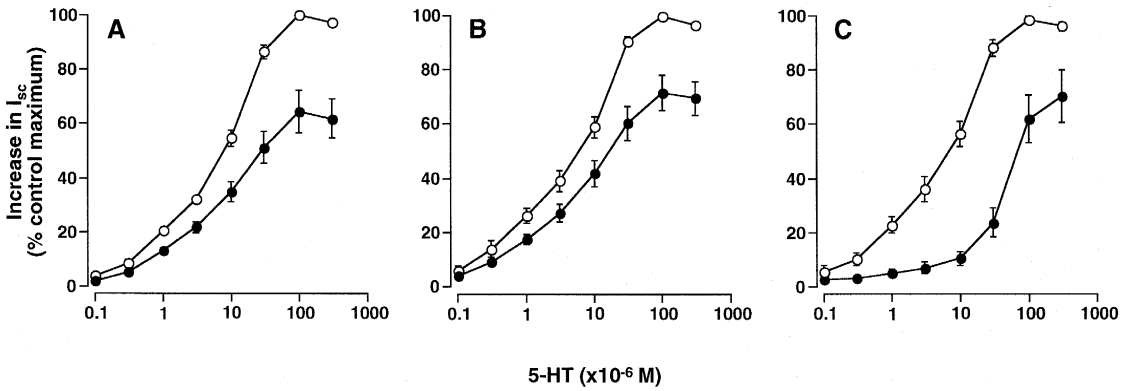

Fig. 2. Effects of tetrodotoxin (10^{-6} M) (A), ramosetron (10^{-7} M) (B) and GR113808 (3×10^{-7} M) (C) on the I_{sc} responses to 5-HT in rat colonic mucosa. Control responses are shown by open circles. The maximal I_{sc} response to 5-HT alone was taken as 100%. The results are the mean ± S.E.M. of 6–8 preparations.

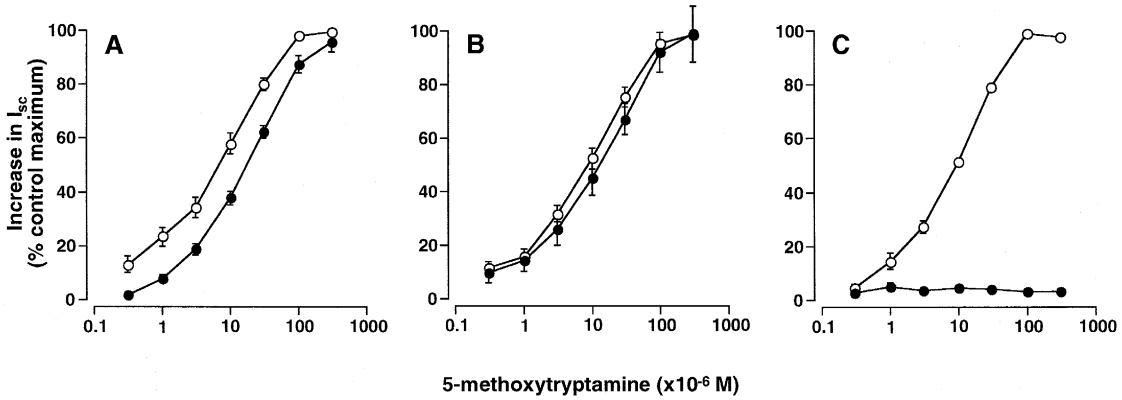

Fig. 3. Effects of tetrodotoxin (10^{-6} M) (A), ramosetron (10^{-7} M) (B) and GR113808 (3×10^{-7} M) (C) on the I_{sc} responses to 5-methoxytryptamine in rat colonic mucosa. Control responses are shown by open circles. The maximal I_{sc} response to 5-methoxytryptamine alone was taken as 100%. The results are the mean ± S.E.M. of 6 preparations.

were examined. Ramosetron (3×10^{-11}–3×10^{-10} M), YM114 (3×10^{-11}–3×10^{-10} M) and granisetron (3×10^{-10}–3×10^{-9} M) concentration-dependently antagonized the I_{sc} responses to 2-methyl-5-HT (Fig. 5). At higher concentrations, each compound caused a decrease in maximal response. Apparent pA_2 values for ramosetron,

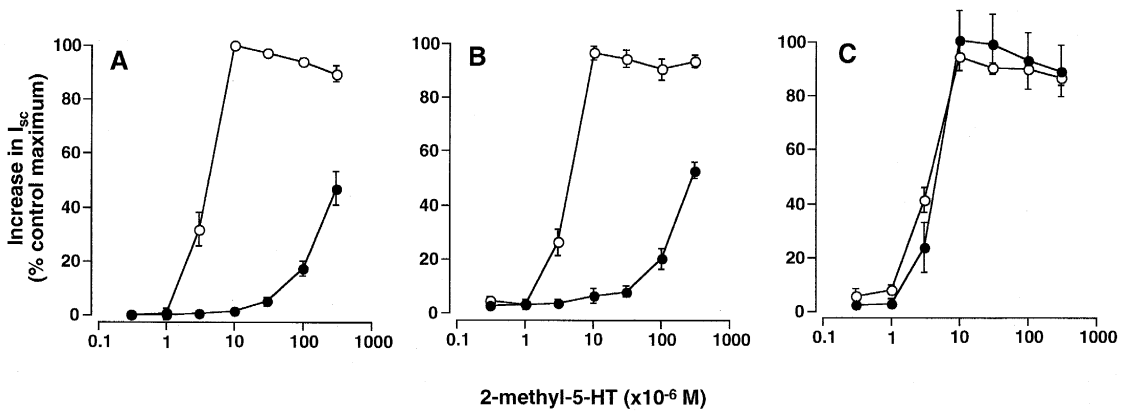

Fig. 4. Effects of tetrodotoxin (10^{-6} M) (A), ramosetron (10^{-7} M) (B) and GR113808 (3×10^{-7} M) (C) on the I_{sc} responses to 2-methyl-5-HT in rat colonic mucosa. Control responses are shown by open circles. The maximal I_{sc} response to 2-methyl-5-HT alone was taken as 100%. The results are the mean ± S.E.M. of 6 preparations.

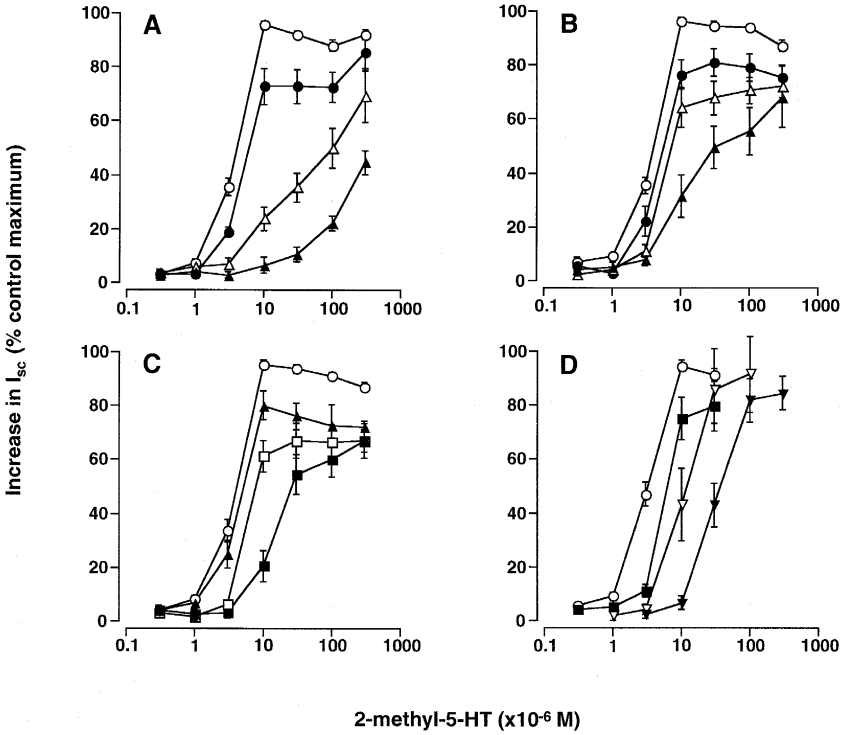

Fig. 5. Effects of ramosetron (A), YM114 (B), granisetron (C) and ondansetron (D) on the I_{sc} responses to 2-methyl-5-HT in rat colonic mucosa. These graphs show concentration-response curves of the effects of each antagonist at concentrations of 3×10^{-11} (●), 10^{-10} (△), 3×10^{-10} (▲), 10^{-9} (□), 3×10^{-9} (■), 10^{-8} (▽), 3×10^{-8} M (▼). Control responses are shown by open circles. The maximal I_{sc} response to 2-methyl-5-HT alone was taken as 100%. The results are the mean ± S.E.M. of 8–24 preparations.

YM114 and granisetron are listed in Table 1. Ondansetron (3×10^{-9}–3×10^{-8} M) produced parallel and concentration-dependent shifts to the right of the concentration-response curves to 2-methyl-5-HT without a decrease in the maximal response (Fig. 5). The slope of the Schild plot of ondansetron did not differ from unity. The rank order of antagonist potency as compared by pA_2 values (Table 1) was ramosetron ≥ YM114 > granisetron > ondansetron. Ramosetron had 26 and 73 times, and YM114 24 and 68 times stronger potency than granisetron and ondansetron, respectively.

3.3. Effects of ramosetron with tetrodotoxin or hexamethonium on responses to 2-methyl-5-HT

Tetrodotoxin (10^{-6} M) and ramosetron (10^{-7} M) equally inhibited the I_{sc} response to 2-methyl-5-HT. Ramosetron with tetrodotoxin did not reduce the I_{sc} response more than either drug alone (Fig. 6A). Hexamethonium (10^{-4} M) partially reduced the response to 2-methyl-5-HT. Ramosetron with hexamethonium did not inhibit I_{sc} response further than ramosetron alone (Fig. 6B). Atropine (10^{-6} M) did not affect the I_{sc} response produced by 2-methyl-5-HT (data not shown).

Table 1
Potencies of 5-HT_3 receptor antagonists in rat colonic mucosa

Compounds	pA_2
YM060	10.43 ± 0.23 [a]
YM114	10.37 ± 0.14 [a]
Granisetron	8.99 ± 0.20 [a]
Ondansetron	8.54 (8.31 ± 9.04)

Values represent the geometric mean ± S.E.M. or 95% confidence limit ($n = 8$). [a] Apparent pA_2 value.

4. Discussion

In rat distal colon, the 5-HT_4 receptor agonist 5-methoxytryptamine increased I_{sc} to 81% of the maximum 5-HT-induced response, while the 5-HT_3 receptor agonists 2-methyl-5-HT and *m*-chlorophenylbiguanide increased I_{sc} to 36% and 33% of the maximum response to 5-HT, respectively. The maximum I_{sc} response to 5-HT decreased to 72% of maximum in the presence of 10^{-7} M ramosetron. In addition, I_{sc} response to 5-HT was remarkably inhibited by 3×10^{-7} M GR113808. Taken together, these results suggest that in the rat distal colon, 5-HT increases I_{sc} through the 5-HT_3 and 5-HT_4 receptors, and that the response mediated by 5-HT_4 receptors is predominant. Furthermore, the effects of 10^{-6} M tetrodotoxin to I_{sc} responses to 5-HT, 2-methyl-5-HT and 5-

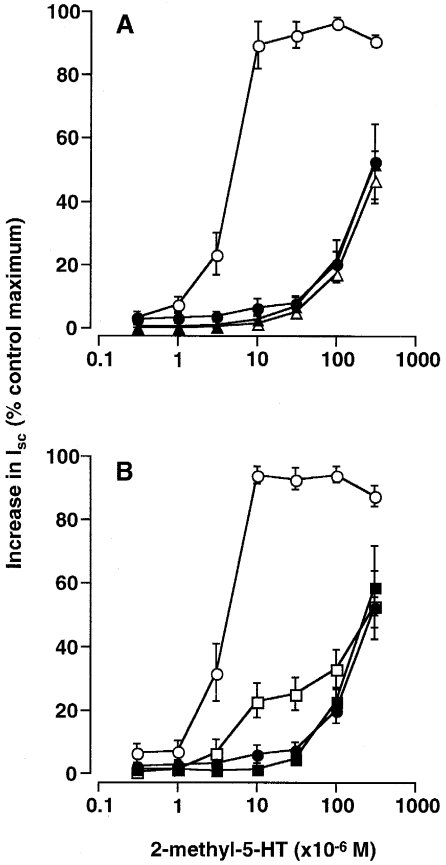

Fig. 6. The effect of ramosetron with tetrodotoxin (A) or with hexamethonium (B) on the I_{sc} responses to 2-methyl-5-HT in rat colonic mucosa: (○) Control; (●) ramosetron, 10^{-7} M; (△) tetrodotoxin, 10^{-6} M; (▲) ramosetron with tetrodotoxin; (□) hexamethonium, 10^{-4} M; (■) ramosetron with hexamethonium. The maximum I_{sc} response to 2-methyl-5-HT alone was taken as 100%. The results are the mean ± S.E.M. of 6 preparations.

methoxytryptamine indicate that the response via 5-HT_3 receptors is neuronal, while the response via 5-HT_4 receptors is non-neuronal. These findings agree with previous reports (Cooke et al., 1991; Budhoo et al., 1996).

Ramosetron and YM114 were more potent than either granisetron or ondansetron for the inhibition of 2-methyl-5-HT-induced I_{sc} responses in the rat distal colon. The pA_2 values in this investigation were in good agreement with those for the inhibition of 5-HT-induced depolarization of rat vagus nerve and pK_i values for the displacement of [^{3}H]GR65630 binding in rat cerebral cortex reported previously (Ito et al., 1995). Moreover, binding study using [^{3}H]ramosetron demonstrated that native 5-HT_3 receptors in rat brain, ileum, colon and cloned rat 5-HT_3 receptors were identical (Akuzawa et al., 1996). These results indicate that 5-HT_3 receptors involved in secretion in the rat distal colon are similar to those in other tissues, and that no intra-species difference in 5-HT_3 receptor could be demonstrated in the rat.

The antagonism of ramosetron, YM114 and granisetron in the rat distal colon was insurmountable, unlike that of ondansetron. This phenomenon was also observed in the rat vagus nerve (Ito et al., 1995). The rate of dissociation of ramosetron and YM114 from 5-HT_3 receptors was reported to be slower than that of ondansetron, whereas that of granisetron was similar (Newberry et al., 1993; Yamano et al., 1994). This insurmountable antagonism of ramosetron and YM114 is therefore assumed to be due to the slow dissociation of the antagonist from the receptor (Elliott et al., 1990). This assumption cannot, however, explain the action of granisetron. Further investigations are needed to clarify the action of granisetron.

We examined the pathway of 2-methyl-5-HT-induced increase in I_{sc}, using ramosetron, tetrodotoxin, hexamethonium and atropine. Tetrodotoxin and ramosetron equally inhibited the I_{sc} response to 2-methyl-5-HT, and there was no additive effect when they were used together. The increase in I_{sc} to 2-methyl-5-HT was partially reduced by hexamethonium, but not by atropine. These results suggest that 2-methyl-5-HT stimulates an increase in I_{sc} by neuronal 5-HT_3 receptors, and that its pathway is, at least in part, mediated via nicotinic receptors.

In conclusion, 5-HT induced an increase in I_{sc} through 5-HT_3 and 5-HT_4 receptors in the rat distal colon. Ramosetron selectively blocked the I_{sc} response mediated by the 5-HT_3 receptor. Furthermore, the 5-HT_3 receptor blocking activity of ramosetron and YM114 is more potent than that of granisetron and ondansetron in rat colonic mucosa.

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