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A dual role of 5-hydroxytryptamine receptor 3 in serotonin induced ion transport in rat distal colon

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Abstract

5-hydroxytryptamine (5-HT)-evoked intestinal secretion can be divided into neural and non-neural pathway. Recently, 5-HT_3 receptor in neural pathway received much attention as a possible target in bowel diseases. The present study aims to investigate the effects of 5-HT_3 receptor in different enteric neural plexus (myenteric plexus and submucosal plexus) on rat colonic ion transport by using rat intact colon and mucosa/submucosa preparations. Ussing chamber and real-time PCR techniques were performed in our present study. Surprisingly, we found that in mucosa/submucosa preparations, 5-HT-induced ΔI_{SC} (change in short-circuit current) was not inhibited, but further increased by pretreatment with 5-HT_3 receptor antagonists, MDL72222 and Tropanyl-3, 5-dimethylbenzoate. And this response was significantly attenuated in the presence of tetrodotoxin (TTX). Conversely, in rat intact colon, 5-HT_3 receptor antagonists significantly inhibited 5-HT-induced ΔI_{SC}. The results from real-time PCR proved the existence of 5-HT_3 receptor in muscularis externa and submucosa. Taken together, 5-HT_3 receptors possess a role of dual regulation on electrolyte secretion in rat distal colon, the neural stimulatory effect of 5-HT_3 receptor in myenteric plexus and the inhibitory effect of 5-HT_3 receptor in submucosal plexus.

Keywords: 5-HT_3 receptor; Ion transport; Neural pathway; Inhibitory; Ussing chamber

1. Introduction

5-HT (5-hydroxytryptamine, serotonin) is an important active enteric amine in the gastrointestinal system. It has been suggested to play a role in the regulation of colonic motor and secretory functions in several species and humans. In mammals, about 60–90% (up to 10 mg in man) of the total amount of 5-HT in the body is in gastrointestinal tract and most of it exists in enterochromaffin (EC) cells (Kellum et al., 1999). 5-HT is secreted from EC cells in response to variety of luminal mechanical and chemical stimulations within the gastrointestinal wall. It is also related to several gastrointestinal dysfunctions such as emesis, motility disorders, diarrhea, and more recently, irritable bowel syndrome (Hoyer et al., 2002). Other major sites of 5-HT are the central nervous system, myenteric nerve system, platelets and so on.

5-HT produces its effects through membrane-bound receptors in different organs. In vitro studies, 5-HT-evoked intestinal secretion was divided into two pathways: neural and non-neural pathway (Budhoo et al., 1996a; Hansen and Skadhauge, 1997; Kiso et al., 1997). The non-neural pathway-mediated stimulatory effect in our previous study was found to be mainly via 5-HT_4 receptor residing at the level of the colonocyte (Ning et al., 2004). while the neural pathway was chiefly mediated by 5-HT_3 receptor (Budhoo et al., 1996a; Kiso et al., 1997). Using intact

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rat distal colon, Budhoo et al. found that specific 5-HT_3 receptor agonist, 2-methyl-5-HT produced a concentration-dependent change in I_{SC} and its action was antagonized by tetrodotoxin (TTX, an inhibitor of neural conduction) (Budhoo et al., 1996a; Kiso et al., 1997), which indicates that the 5-HT_3 receptor produces a stimulatory effect in neural pathway.

Recently, the neural 5-HT_3 receptor received much attention as a possible target in bowel diseases (Michel et al., 2005). And 5-HT_3 receptor antagonists have been used to relieve symptoms associated with diarrhea-predominant irritable bowel syndrome (Hicks et al., 2002). But the exact mechanism underlying 5-HT_3 receptor neurally mediated ion transports in colon is still unclear.

The enteric nervous system (ENS) is the part of the nervous system that directly controls the gastrointestinal system. The neurons of the ENS are collected into two types of ganglia: myenteric (Auerbach's) and submucosal (Meissner's) plexuses. Myenteric plexuses are located between the inner and outer layers of the muscularis externa, while submucosal plexuses are located in the submucosa (Crone et al., 2003). The present study aims to investigate the effects of 5-HT_3 receptors in different enteric neural plexus (myenteric plexus and submucosal plexus) on rat colonic ion transport by using intact colon and mucosa/submucosa preparations.

2. Materials and methods

2.1. Animals

Animal protocols followed guidelines established by the NIH and were approved by Animal Care and Use Committee, Capital Medical University. Adult male Sprague–Dawley rats (Laboratory Animal Services Center, Capital Medical University) ranging in weight from 200 to 300 g had free access to standard rodent laboratory food and water until the day of the experiment. The animals were killed by cervical dislocation. The distal colon was removed and defined as the ~7 cm-long segment proximal to the lymph node (typically situated 3 cm apart from the anus). Then the distal colon was divided into four segments, termed DC_1 (adjacent to the lymph node), DC_2, DC_3 and DC_4, respectively. Preliminary results indicated that the differences existed in the four segments (Yang et al., 2006). But the responses of DC_3 and DC_4 to 5-HT were similar and stable. Therefore, in the present study rat DC_3 and DC_4 were taken to investigate the roles of 5-HT_3 receptors. Every DC_3 and DC_4 was cut along the mesenteric border into a flat sheet and flushed with ice-cold Krebs–Henseleit solution (K–HS) containing (in mmol/l): 117 NaCl, 4.7 KCl, 1.2 $MgCl_2 \cdot 6H_2O$, 1.2 NaH_2PO_4, 25 $NaHCO_3$, 2.5 $CaCl_2 \cdot 2H_2O$, 11.1 D-glucose. The tissue was pinned flat with the mucosal side down in a Sylgard-lined petri dish containing ice-cold oxygenated reparation.

2.2. Reagents

5-hydroxytryptamine (5-HT), indomethacin, MDL-72222 (Tropanyl 3,5-dichlorobenzoate), GR113808 ([1-[2-(methylsulfonyl-amino) ethyl]-4-piperidinyl] methyl 1-methylindole-3-carboxylate) and tetrodotoxin (TTX) were obtained from Sigma Chemical Company (St. Louis, MO, USA). Tropanyl-3, 5-dimethylbenzoate was purchased from Tocris Cookson Inc. (Ellisville, Missouri, USA). Stock solutions of some chemicals (indomethacin, MDL-72222, GR113808, Tropanyl-3, 5-dimethylbenzoate) were dissolved in dimethyl sulfoxide (DMSO). Final DMSO concentrations never exceeded 0.1% (vol/vol). Preliminary experiments indicated that the vehicle did not alter any baseline electrophysiological parameters.

2.3. Ussing chamber experiments

Flat sheets of mucosa/submucosa preparation or intact colon were mounted in modified Ussing chambers with a cross-sectional area being 0.5 cm^2. The mucosal and serosal surfaces of tissue were bathed with 5 ml K–HS recirculated from a reservoir maintained at 37 °C and bubbled with 95% O_2 and 5% CO_2 to maintain the pH of the solution at 7.4. Drugs were added directly to the apical or basolateral side of epithelium. Responses were recorded continuously. Transepithelial potential difference for every colonic mucosa was measured with the Ag/AgCl reference electrodes (Physiologic Instruments, P2020S) connected to a preamplifier that was in turn connected to a voltage-clamp amplifier VCC MC6 (Physiologic Instruments, San Diego, CA, USA). The change in short-circuit current (ΔI_{SC}) was calculated as difference between before and after simulation, I_{SC} was normalized as current per unit area of epithelial ($\mu A/cm^2$), which allowed the curve area for 15 min to be calculated (μA min).

2.4. RNA extraction and preparation of cDNA

Two preparations from 9 rats were collected in phosphate buffer solution (PBS), which had been treated with 0.1% diethyl pyrocarbonate (DEPC-PBS), namely the intact colon and mucosa/submucosa preparations. Preparations were cut open along the mesenteric border, and the contents were flashed out with DEPC-PBS and immediately snap frozen in liquid nitrogen. RNA from gut tissue was harvested using the Trizol RNA purification system (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions.

2.5. Real-time polymerase chain reaction

Real-time PCR was used to quantify mRNA encoding 5-HT_3 receptors in the intact and mucosa/submucosa preparations of rat distal colon. The expression of 5-HT_3 was normalized to that of β-actin, a housekeeping gene that is not thought to be subject to regulation. Transcripts encoding 5-HT_3 receptors in samples of rat colon were comparatively quantified by real-time PCR with the Brilliant SYBR Green QPCR Master Mix kit (Stratagene, La Jolla, CA, USA) using a Light Cycler instrument (Stratagene).

Amplifications were performed in a final volume of 20 μl of a commercial reaction mixture according to the manufacturer's instructions. The primers for the amplification of cDNA encoding β-actin and 5-HT_3 receptors were used at a final concentration of 0.2 μmol/l. 0.25 μl of the cDNA prepared from tissue was added to the mixture. Data were analyzed with computer assistance using the MxPro QPCR software (version 3.0, Mx3000P system,

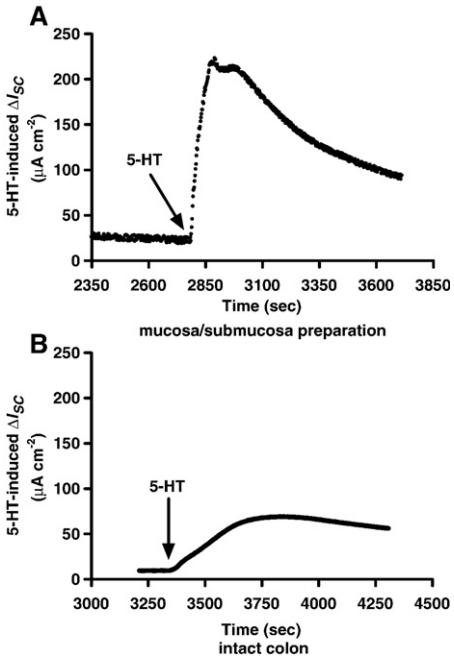

Fig. 1. 5-HT-induced I_{SC} responses in rat mucosa/submucosa preparations (A) and intact colon (B). Even at 100 μmol/l, 5-HT-induced I_{SC} responses in rat intact colon were smaller than that (10 μmol/l) in rat mucosa/submucosa preparation.

Stratagene). Primer sequences were: β-actin forward primer: 5′-TTC AAC ACC CCA GCC ATG T-3′, reverse primer: 5′-GTG GTA CGA CCA GAG GCA TAC A-3′; 5-HT_3 receptor forward primer 5′-TGC ATA CCA TCC AGG ACA TCA-3′, reverse primer: 5′-CTC TTG TCC GAC CTC ACT TCT TC-3′.

2.6. Statistical analysis

The data were expressed as means ± standard error of mean (S.E.M.). "*n*" refers to the number of tissue preparations. Comparisons between groups of data were made via Student's paired or unpaired *t*-test. *P*-values <0.05 were considered statistically significant.

3. Results

3.1. 5-HT-induced I_{SC} responses in rat intact colon and mucosa/submucosa preparations

To investigate the role of 5-HT_3 receptor in regulation of ion transport, rat intact colon and mucosa/submucosa preparations were used respectively. In both of the preparations, indomethacin (10 μmol/l), a cyclooxygenase (COX) inhibitor, was routinely added to the basolateral side to abolish the effects of endogenous prostaglandins. 5-HT and antagonists of 5-HT receptors were added to the basolateral sides of the tissues in the present study since basolateral addition, but not apical application, of 5-HT was able to elicit an increase in I_{SC}. (Ning et al., 2004) After equilibration for 30 min, 5-HT (10 μmol/l)-produced I_{SC} increase was 1240.0 ± 91.3 μA min (n=12) in rat mucosa/submucosa preparations. However, in intact colon, 5-HT, in 10 μmol/l,-induced I_{SC} response was very small and can be ignored (data not shown), and 5-HT, in

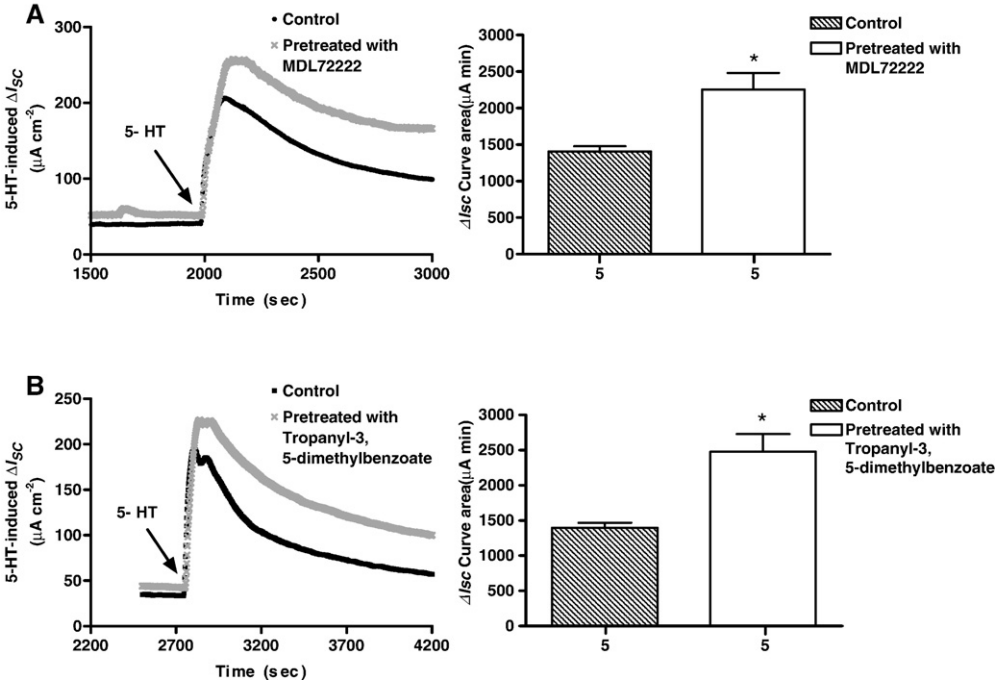

Fig. 2. Effect of 5-HT_3 receptor antagonists on 5-HT-induced I_{SC} responses in rat distal colon mucosa/submucosa preparations. (left) Representative I_{SC} recording with arrows indicating the time for the basolateral application of 5-HT (10 μmol/l) to rat mucosa/submucosa preparations basolateral pretreated with MDL72222 (10 μmol/l, A) or Tropanyl-3, 5-dimethylbenzoate (10 μmol/l, B), respectively. (right) Summary of the effects of MDL72222 (10 μmol/l, A) and Tropanyl-3, 5-dimethylbenzoate (10 μmol/l, B) on 5-HT-induced ΔI_{SC}. Values are means ± S.E.M.; *, P<0.05.

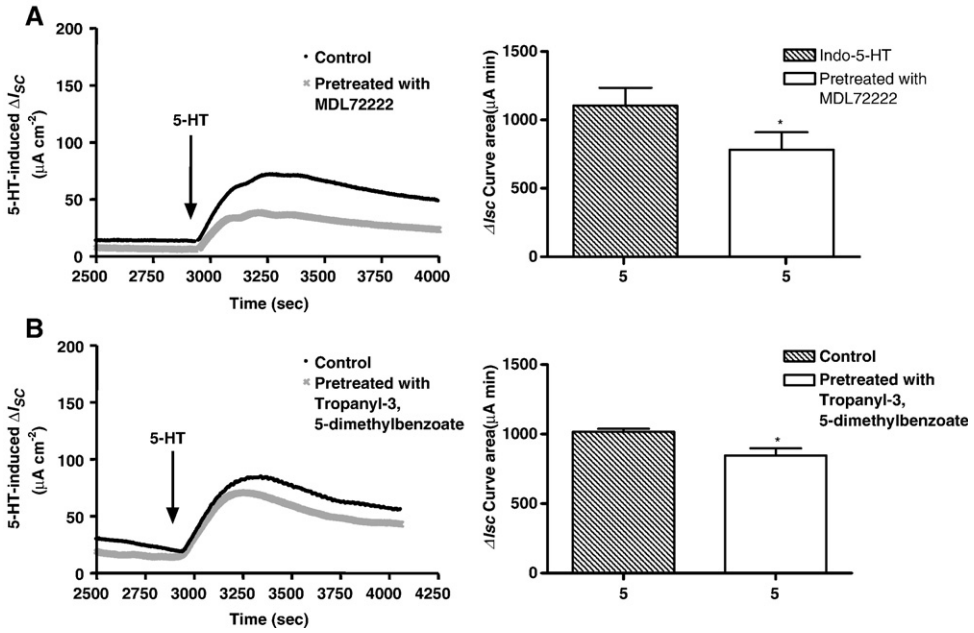

Fig. 3. Effect of 5-HT_3 receptor antagonists on 5-HT-induced I_{SC} responses in rat intact colon preparations. (left) Representative I_{SC} recording with arrows indicating the time for the basolateral application of 5-HT (10 μmol/l) to rat intact colon preparations basolateral pretreated with MDL72222 (10 μmol/l, A) or Tropanyl-3, 5-dimethylbenzoate (10 μmol/l, B), respectively. (right) Summary of the effects of MDL72222 (10 μmol/l, A) and Tropanyl-3, 5-dimethylbenzoate (10 μmol/l, B) on 5-HT-induced ΔI_{SC}. Values are means±S.E.M.; *, $P<0.05$.

100 μmol/l,-induced change in I_{SC} was 997.0±83.5 μA min ($n=9$). (Fig. 1)

3.2. Effects of 5-HT_3 receptor antagonists on 5-HT-induced I_{SC} responses in rat intact colon and mucosa/submucosa preparations

The pharmacological profile of 5-HT_3 receptor in rat intact colon and mucosa/submucosa preparation was evaluated by using specific antagonists (MDL72222 and Tropanyl-3,5-dimethylbenzoate) to 5-HT_3 receptor.

In rat mucosa/submucosa preparations, pretreatment with 5-HT_3 receptor antagonists MDL72222 (10 μmol/l) and Tropanyl-3, 5-dimethylbenzoate (10 μmol/l) for 5 min did not inhibit, but increased 5-HT (10 μmol/l)-induced ΔI_{SC} from 1406.0± 72.7 μA min and 1395.0±73.7 μA min to 2255.0±227.0 μA min ($n=5$, $P<0.05$, Fig. 2A) and 2479.0±249.5 μA min ($n=5$,

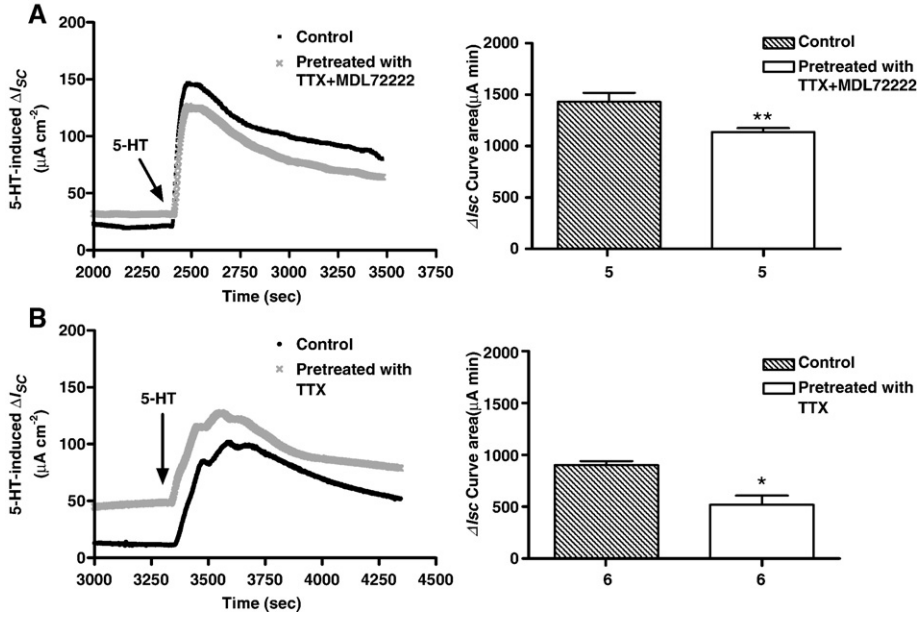

Fig. 4. Role of neural pathway in 5-HT-induced I_{SC} responses. (left) Representative I_{SC} recording with arrows indicating the time for the basolateral application 5-HT (10 μmol/l) to rat mucosa/submucosa preparations basolateral pretreated with TTX (1 μmol/l) and MDL72222 (10 μmol/l, A) or rat intact colon preparations basolateral pretreated with TTX (1 μmol/l, B), respectively. (right) Summary of the effects of TTX (1 μmol/l) and MDL72222 (10 μmol/l, A) or TTX (1 μmol/l, B) on 5-HT-induced ΔI_{SC} in rat mucosa/submucosa or intact colon preparations, respectively. Values are means±S.E.M.; *, $P<0.05$; **, $P<0.01$.

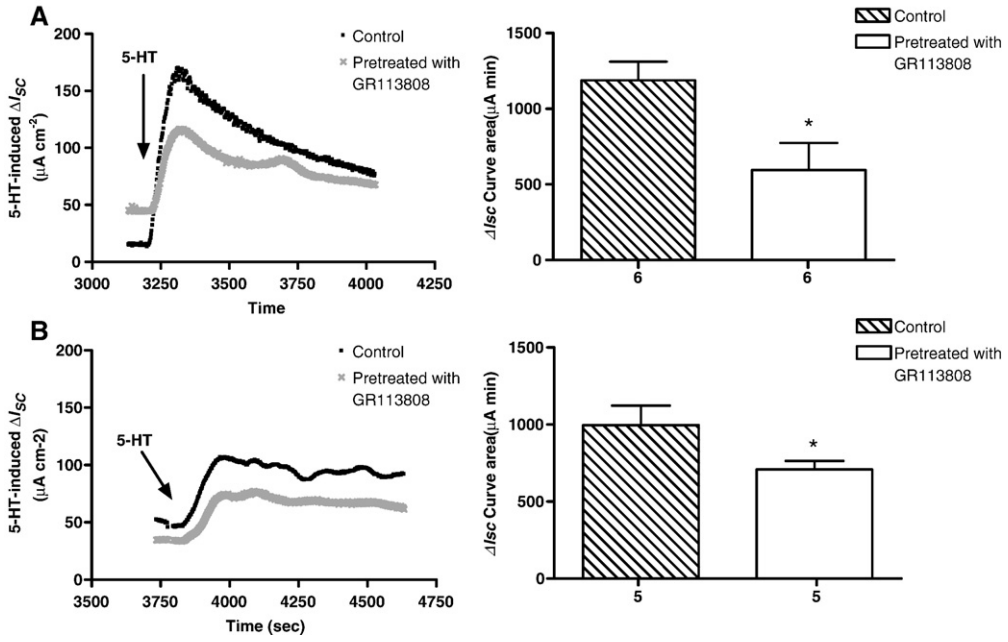

Fig. 5. Effect of 5-HT_4 receptor antagonist on 5-HT-induced I_{SC} responses in rat mucosa/submucosa and intact colon preparations. (left) Representative I_{SC} recording with arrows indicating the time for the basolateral application of 5-HT to rat mucosa/submucosa (A) and intact colon (B) preparations basolateral pretreated with GR 113808 (0.1 μmol/l), respectively. (right) Summary of the effects of GR 113808 (0.1 μmol/l) on 5-HT-induced ΔI_{SC} in rat mucosa/submucosa (A) and intact colon (B) preparations. Values are means±S.E.M.; *, $P<0.05$.

$P<0.05$, Fig. 2B), respectively. Whereas, in rat intact colon, both of the 5-HT_3 receptor antagonist MDL72222 (10 μmol/l) and Tropanyl-3, 5-dimethylbenzoate (10 μmol/l) significantly decreased 5-HT (100 μmol/l)-induced I_{SC} from 1105.0±130.0 μA min and 1017.0±21.8 μA min to 782.5±128.0 μA min ($n=5$, $P<0.05$, Fig. 3A) and 846.7±50.4 μA min ($n=5$, $P<0.05$, Fig. 3B), respectively.

3.3. Effects of neural pathways on 5-HT-induced I_{SC} responses in rat intact colon and mucosa/submucosa preparations

It has been reported that 5-HT-induced I_{SC} responses is mainly neurally mediated by the 5-HT_3 receptor (Budhoo et al., 1996a). To study the role of enteric neural pathway, tetrodotoxin (TTX, 1 μmol/l), an inhibitor of neural conduction, was chosen. In rat mucosa/submucosa preparations basolateral pretreated with TTX, the increased effect of 5-HT_3 antagonist on 5-HT-induced responses was absent. On the contrary, the inhibitory effect was observed, 5-HT (10 μmol/l)-induced I_{SC} was significantly reduced by MDL72222 (10 μmol/l, basolateral side) from 1429.0±89.7 μA min to 1135.0±41.3 μA min ($n=5$, $P<0.01$, Fig. 4A). In rat intact colon, pretreated with TTX, 5-HT (100 μmol/l)-induced current was decreased from 900.9±39.5 μA min to 518.4±90.6 μA min ($n=6$, $P<0.05$, Fig. 4B).

3.4. Effects of 5-HT_4 receptor antagonists in rat intact colon and mucosa/submucosa preparations

Pretreated with 5-HT_4 antagonist GR113808 (0.1 μmol/l, basolateral side), 5-HT (10 μmol/L)-induced ΔI_{SC} was significantly inhibited by about 50% (from 1188.0±122.5 μA min to 595.2±178.6 μA min, $n=6$, $P<0.05$, Fig. 5A) in rat mucosa/submucosa preparations and 28.84% (from 995.2±127.9 μA min to 707.6±55.3 μA min, $n=5$, $P<0.05$, Fig. 5B) in rat intact colon.

3.5. The mRNA expression of 5-HT_3 receptor in different preparations of rat colon

In rat gastrointestinal tract, 5-HT_3 receptors were found in numerous myenteric and submucosal neurons and were abundant in fibers within the myenteric plexus (Thompson and Lummis, 2006). It has been reported that the 5-HT_3 receptor subunits may be the same (homopentameric 5-HT_{3a} receptors) or different (heteropentameric receptors, usually comprising of 5-HT_{3a} and 5-HT_{3b} receptor subunits), with the latter having a number of distinct properties (Siriwardena et al., 1993). Therefore, we chose the 5-HT_{3a} receptor subunit as the marker of the 5-HT_3 receptor. Real-time PCR was used to compare the rat distal colonic mRNA expressions of 5-HT_3 receptor in intact colon, mucosa/submucosa and mucosa preparations in the present study. The relative mRNA quantities of 5-HT_{3a} receptor subunit were normalized to the β-actin in each preparation. It is obvious that the mRNA expression of 5-HT_{3a} receptor subunit exists in each layer of rat distal colon. The rank order expression of 5-HT3a receptor subunit was intact colon>mucosa/submucosa preparations (M+S)>mucosa preparations (M).

4. Discussion

Generally, the Ussing chamber voltage-clamp model is demonstrated as a measure of electrolyte transport. The secretory response of anion is reflected by an increase in short-circuit current

(I_{SC}). Then, in the present study, Ussing chamber was used to measure the secretory responses induced by 5-HT and the effects of 5-HT receptor antagonists. The classic study by Budhoo et al. demonstrated that 5-HT-stimulated mucosal electrolyte transport is mediated by a neural and non-neural mechanism (1996a). The investigators also found that in intact colon the stimulatory effect of 5-HT on gastrointestinal tract mediated by neural pathway is regulated via the 5-HT_3 receptor (Siriwardena et al., 1991, 1993). In our present study, a similar stimulatory role of 5-HT_3 receptor was found in rat intact colonic preparation for 5-HT_3 receptor antagonists inhibited 5-HT-induced ΔI_{SC}. Moreover, after incubation with a neuronal Na^+ channel blocker, TTX, 5-HT-induced ΔI_{SC} in rat intact colon was slightly minished. Arcuni et al. reported that TTX was able to abolish 5-HT_3 receptor agonist, 2-methyl-5-HT,-induced changes in I_{SC} due to its effects of chemically blocking an intact intrinsic innervation (2000). These results suggest that the neural stimulatory effect of 5-HT probably comes from the activated 5-HT_3 receptors in enteric plexus. However, one striking find in rat mucosa/submucosa preparations was the inhibitory effect of 5-HT_3 receptor on rat colonic secretion. Results from the present study show that 5-HT_3 receptor antagonists, MDL72222 and Tropanyl-3, 5-dimethylbenzoate, increased, but not decreased 5-HT-induced ΔI_{SC} in rat mucosa/submucosa preparations and the effects were significantly attenuated in the presence of TTX. It illustrates a possible mechanism of inhibitory effect on anion secretion depending on the submucosal plexus mediated by 5-HT_3 receptor. The inhibitory effect of 5-HT_3 receptor on 5-HT-induced secretion in rat distal colon has never been reported although Ishizawa reported an inhibitory action of 5-HT on spontaneous propulsive activities depending on an inhibitory neurotransmitter-mediated endogenous prostaglandins release from the circular muscle cells (1996).

Our results from real-time PCR provide the evidences for the mRNA expressions of 5-HT_{3a} receptors in both intact colon and mucosa/submucosa preparations. The rank order of 5-HT_{3a} receptor subunit expression, intact colon > mucosa/submucosa preparation (M+S) > mucosa preparation (M), suggested that the 5-HT_{3a} receptor mRNA existed in both muscularis externa and submucosa. It has been demonstrated that 5-HT_3 receptor-like immunoreactivity occurs in enteric neurons of all layers in the rat colon (Mazzia et al., 2003). Michel et al. also found the positive staining for 5-HT_{3a} and 5-HT_{3b} receptor in all neurons of the human submucous plexus (2005). These results provide possibility to our hypothesis got from Ussing chamber experiments, in neural pathway the stimulatory effect of 5-HT on electrolyte secretion owing to the activated 5-HT_3 receptor in myenteric plexus and the inhibitory effect of 5-HT being mediated by 5-HT_3 receptor in the submucosal plexus.

On the other hand, previous studies from our laboratory have shown that 5-HT elicited an ion transport by acting directly on the colonic mucosa via 5-HT_4 receptors (Ning et al., 2004). In the present study, we further investigated whether or not the 5-HT_4 receptor could also mediate an stimulatory I_{SC} response with the mucosa/submucosa preparations. Expectably, the effects of 5-HT_4 receptor antagonist on 5-HT-induced ion transport in intact colon and mucosa/submucosa preparations were similar to that in colonic mucosa. It is consistent with the reports that 5-HT-induced non-neural ion transport is mediated by 5-HT_4 receptor in rat distal colon and human jejunal mucosa (Budhoo et al., 1996b; Ning et al., 2004).

Summing up, 5-HT_3 receptors possess a role of dual regulation on electrolyte secretion in rat distal colon, the neural stimulatory effect of 5-HT_3 receptor in myenteric plexus and the inhibitory effect of 5-HT_3 receptor in submucosal plexus. This leads to an increase in our understanding of the role of 5-HT in normal gastrointestinal (GI) physiology. It is well known that 5-HT_3 and 5-HT_4 receptors play important roles in regulation of 5-HT on enteric water and electrolyte flux (Budhoo et al., 1996a). Up to now, both 5-HT agonists and antagonists have proven clinical efficacy to irritable bowel syndrome (irritable bowel syndrome with diarrhea, irritable bowel syndrome with constipation), though in both cases, the large number needed to treat suggests that improved ways are needed to target and characterize patients who are likely to respond (Spiller, 2007). Our amazing finding that 5-HT_3 receptors play dual roles in regulation of gastrointestinal secretion may be particularly significant in relation to the pathogenesis of various gastrointestinal dysfunctions and provide the clue for diagnosis and treatment to different kind of patients (Tonini and Pace, 2006).

Acknowledgments

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