Colonic submucosal 5-HT₃ receptor-mediated somatostatin-dependent secretoinhibitory pathway is suppressed in water-immersion restraint stressed rats

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1. Introduction

5-Hydroxytryptamine (5-HT), a neuroendocrine transmitter, is abundant in the gastrointestinal tract. As a potent paracrine secretagogue in all parts of the small intestine and colon in all studied species, including humans (Engelmann et al., 2006; Hansen and Skadhauge, 1997; Safsten et al., 2006; Tuo and Isenberg, 2003), 5-HT produces its effects through different membrane-bound receptors in the gut (5-HT₁A, 5-HT₁B, 5-HT₂, 5-HT₄ and 5-HT₇) (nomenclature follows Alexander et al., 2009), and mediates secretomotor effect mainly via 5-HT₃ receptors residing at the level of the colonocyte in the nonneural pathway (Goldhill et al., 1998; Ning et al., 2004). In the neural pathway, the activation of 5-HT₃ receptors in the submucosal plexus can suppress 5-HT-induced rat colonic ion secretion by increasing submucosal somatostatin release (Yang et al., 2010).

It is well-recognized that 5-HT and its receptors play important roles in the pathology of intestine dysfunction (Crowell, 2004; Gershon and Tack, 2007; Wood, 2001). Various types of psychological and physical stress have impacts on intestinal mucosal functions, including secretion and the epithelial barrier (Gareau et al., 2008), roles in the pathology of inflammatory diseases and functional disorders of the gastrointestinal tract. Acute stressors are able to increase the baseline chloride secretion in the human jejunum and rat colon by a mechanism involving cholinergic nerve stimulation (Barclay and Turnberg, 1987; Saunders et al., 1997). Additionally, in vivo studies suggest that endogenous 5-HT is one of the substances that mediate the stress-induced responses of gastrointestinal function, such as defecation and diarrhea, and that the effects of 5-HT are mediated by the peripheral 5-HT₃ receptors (Funatsu et al., 2007; Miyata et al., 1992). However, the exact regulatory mechanism of 5-HT-mediated colonic ion transport in stress remains unclear.

5-HT₃ receptor antagonists have been shown to slow down colonic transit, increase fluid absorption, and then improve symptoms in some diarrhea-predominant irritable bowel syndrome patients (Camilleri et al., 2001). Further understanding of the 5-HT₃ receptor

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regulatory pathways in gastrointestinal secretion can provide insights into the etiology and treatment of gastrointestinal disorders/diseases. In the present study, we aim to investigate the mechanism underlying the effect of acute stress on the 5-HT$_3$ receptor-mediated secretoinhibitory pathway in the rat distal colon.

2. Materials and methods

2.1. Animals and water immersion-restraint stress model

The animal use protocol was approved by the Animal Care and Use Committee of Capital Medical University and met NIH guidelines. Adult male Sprague-Dawley rats (Laboratory Animal Services Center, Capital Medical University) ranging from 200–250 g had free access to standard rodent laboratory food and water, and were allowed to acclimate one week before starting the experiments. The rats were randomly divided into a control group and a water-immersion restraint stress (WIRS) group. In the WIRS group, the rats were assigned to receive water-immersion restraint stress for 2 h. They were tied to a wooden board, and their entire bodies, except for the heads, were immersed vertically to the level of the xiphoid process in a water bath maintained at 19±1 °C for 2 h, as previously described (Konturek et al., 2002). The animals remaining undisturbed in their home cages served as unstressed controls. The rats were all sacrificed immediately by cervical dislocation after water immersion-restraint stress; the distal colons were rapidly removed and were subjected to short-circuit current ($I_{sc}$) recording, detection of receptor expression, and radioimmunoassay.

2.2. Tissue preparation

The distal colon was removed and defined as the ~7-cm long segment proximal to the lymph node (typically situated 3 cm from the anus). The distal colon was then divided into four segments, termed DC1 (adjacent to the lymph node), DC2, DC3 and DC4. The preliminary results indicated that the responses to 5-HT were different in the four segments; however, the responses in DC3 and DC4 were more reliable and consistent (Yang et al., 2006). Thus, the tissues adjacent to the DC3 and DC4 segments were used in the present study. Each segment was cut along the mesenteric border into a flat sheet and was flushed with ice-cold oxygenated K-HS (Krebs–Henseleit solution) containing (in mM): 117 NaCl, 4.7 KCl, 1.2 MgCl$_2$·6H$_2$O, 1.2 NaH$_2$PO$_4$, 25 NaHCO$_3$, and 2.5 CaCl$_2$·2H$_2$O. The tissue was pinned flat with the mucosal side down in a petri dish containing ice-cold oxygenated K-HS. The serosa and the muscle layers were carefully removed under a dissecting microscope to obtain the mucosa/submucosa preparations. To detect the expression of mucosal 5-HT$_4$ receptors, somatostatin receptor 2 (sst$_2$ receptor), and submucosal 5-HT$_3$ receptor, mucosae were isolated from the submucosa.

2.3. Short-circuit current measurement

The short-circuit current was measured in vitro in Ussing chambers. A flat sheet of the colonic mucosa/submucosa was mounted between two halves of a modified Ussing chamber, in which the total cross-sectional area was 0.5 cm$^2$. Both the mucosal and basolateral sides were bathed with 5 ml K-HS, gassed with 95% O$_2$ and 5% CO$_2$, and maintained at 37 °C. The tissue was continuously voltage-clamped to zero potential difference by the application of external current, with compensation for fluid resistance. The transepithelial resistance ($\Omega$ cm$^2$) was measured by altering the membrane potential stepwise (~0.1 mV) and applying Ohm’s law. The baseline value of the electrical parameters was determined as the mean over the 3 min immediately prior to drug administration. The tissues were allowed to rest for approximately 30 min to stabilize the $I_{sc}$ prior to the addition of drugs. The transepithelial potential difference for each preparation was measured with Ag/AgCl reference electrodes (Physiologic Instruments, San Diego, CA, USA; P2020S) connected to a preamplifier that was, in turn, connected to a voltage-clamp amplifier (Physiologic Instruments, San Diego, CA, USA; VCC M6). The change in the short circuit current (Δ$I_{sc}$) was calculated on the basis of the value before and after the stimulation and was normalized as the current per unit area of epithelium ($\mu$A/cm$^2$), which allowed the area under the curve for 15 min to be calculated ($\mu$A min).

2.4. RNA extraction and cDNA synthesis

The total RNA was extracted from one part of the mucosa and submucosa preparations, using the Trizol RNA purification system (Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s instructions. The total RNA was stored at −80 °C for later use. The first strand cDNA was synthesized following the protocol of the superscript first-strand synthesis system for RT-PCR (Invitrogen), and was stored at −20 °C for future polymerase chain reaction (PCR).

2.5. Real-time PCR

Real-time PCR was employed to quantify the gene expression of mucosal 5-HT$_4$ receptor, sst$_2$ receptor, and submucosal 5-HT$_3$ receptor in the distal colon of the rats. The expression of β-Actin was used as an internal control. Transcripts of the colonic preparations in the WIRS and control rats 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). The amplifications were performed in a final volume of 20 μl of a commercial reaction mixture according to the manufacturer’s instructions. The specific primers were designed according to GenBank and are listed in Table 1. The primers were used at a final concentration of 0.2 μM. The data were analyzed with computer assistance using the MxPro QPCR software (version 3.0, Mx3000P system, Stratagene).

2.6. Western blot analysis

In addition to the part of tissue to be used for real-time PCR, the other part of the mucosa and submucosa layer samples from the same rats were homogenized in 300 μl cold lysis buffer (1% Nonidet P-40, 10 mM Tris–HCl, pH 8.0, 150 mM NaCl, 0.1% SDS, 1 mM EDTA, 5 μg/ml leupeptin, 5 μg/ml aprotinin, 1 mM PMSF, 0.5% deoxycholic acid, and 1 mM sodium orthovanadate, all purchased from Sigma). The total tissue homogenates were sonicated for complete dissolution followed by centrifugation at 12,000 rpm for 30 min at

<table>
<thead>
<tr>
<th>Primer</th>
<th>GenBank accession number</th>
<th>Primer sequence</th>
</tr>
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<tbody>
<tr>
<td>β-Actin</td>
<td>NM031144</td>
<td>Forward: 5′-TTC AAC ACC CCA GCC ATG T-3′&lt;br&gt;Reverse: 5′-GTC GTA CGA CCA GAG GCA TAC A-3′</td>
</tr>
<tr>
<td>5-HT$_4$ receptor</td>
<td>NM024394</td>
<td>Forward: 5′-TGC ATA CCA TCC AGG ACA TCA-3′&lt;br&gt;Reverse: 5′-CTC TGG TCC GAC CTC ACT TCT TC-3′</td>
</tr>
<tr>
<td>5-HT$_3$ receptor</td>
<td>NM012853</td>
<td>Forward: 5′-GCT GGC TCA TTC CCA TGT TT-3′&lt;br&gt;Reverse: 5′-CAA CTA TGC CGA TGT TGC A-3′</td>
</tr>
<tr>
<td>Somatostatin receptor 2</td>
<td>NM019348</td>
<td>Forward: 5′-TTG ACC GTG ATG AGC ACG G-3′&lt;br&gt;Reverse: 5′-ACA GAC ACC GAC GAG ACA TGC-3′</td>
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4 °C. The supernatant was collected, and its protein level was quantified by the BCA assay according to the manufacturer’s protocol (Thermo, Rockford, IL, USA). A total of 100–200 μg protein samples were separated by 10% SDS/polyacrylamide gel electrophoresis (SDS/PAGE) and electroblotted onto a nitrocellulose membrane (NC membrane, Millipore); then, the membrane was blocked in TBS (20 mM Tris-Cl, pH 7.4, containing 150 mM NaCl, and 2.68 mM KCl) with 10% non-fat milk for 1 h at room temperature. The blot was incubated overnight at 4 °C with primary antibodies for the 5-HT3 receptor (Santa Cruz/sc-28958, 1:400 dilution), 5-HT4 receptor (Santa Cruz/sc-13106, 1:300 dilution), sst2 receptor (Santa Cruz/sc-11606, 1:200 dilution), GAPDH (Sigma/G9545, 1:10,000 dilution) or β-Actin (Sigma/AS5060, 1:10,000 dilution). After washing with TBST (TBS containing 0.05% Tween-20), the blot was incubated with the appropriate secondary antibodies (goat anti-rabbit IgG, Rockland/16747, or donkey anti-goat IgG, Rockland/18246, 1:10,000 dilution) for 1 h at room temperature. The blot was finally washed with TBST, scanned by infrared rays with the Odyssey Infrared Imager (LI-COR, Nebraska, USA), and semi-quantified using the Odyssey software (version 1.2). β-Actin or GAPDH was used as the internal control.

2.7. Measurement of somatostatin release

The rat colonic mucosa/submucosa tissues were incubated in tubes containing 1 ml K-HS, gassed with 95% O2 and 5% CO2, and maintained at 37 °C for 30 min for equilibration. The tissues were pretreated with vehicle (0.9% NaCl) or 5-HT3 receptor antagonists for 10 min before adding 5-HT (10 μM, 10 min). At the end of the incubation, the tissue in each tube was removed and underwent protein quantization. All samples in the tubes were collected and stored in liquid nitrogen for subsequent somatostatin measurement. The K-HS was used as negative control. The somatostatin levels of the samples were measured by a commercial radioimmunoassay (RIA) kit (Beijing Sinouk Institute of Biological Technology, Beijing, China).

2.8. Drugs

5-HT, indomethacin, MDL72222 (Tropanyl3, 5-dichlorobenzoate), and CYN154806 (CYANAMID154806) were obtained from Sigma Chemical Company (St. Louis, MO, USA). Stock solutions of indomethacin and MDL72222 were dissolved in DMSO (dimethyl sulphoxide), with final DMSO concentrations never exceeding 0.1% (vol/vol). Others were dissolved in aqueous stock solution.

2.9. Statistical analysis

The values are presented as means ± S.E.M. (standard error of mean); “n” refers to the number of samples. The statistical analysis was conducted using a paired or non-paired t-test. The statistics and graphs were generated using GraphPad Prism, version 4.0 (GraphPad Software Inc., San Diego, CA, USA.). Significance was declared at P < 0.05.

3. Results

3.1. Basic characteristics of electrophysiology and 5-HT-induced colonic secretion in mucosa/submucosa preparations after water immersion-restraint stress

After an equilibration time of 30 min, the baseline \( I_{SC} \) of the mucosa/submucosa preparations in WIRS rats, 56.03 ± 6.01 μA/cm², was significantly higher than that in the control, 37.28 ± 6.21 μA/cm² (n = 11, P < 0.05, Fig. 1 A1). The transepithelial resistance (Rte) in WIRS rats, 56.50 ± 3.40 Ω cm², was obviously lower than that in the control rats, 72.83 ± 6.93 Ω cm² (n = 11, P < 0.05, Fig. 1 A2). Endogenous prostaglandins are reported to be released during tissue preparations (Park et al., 2006) and immobilization stress (Castagliuolo et al., 1996), and the cyclooxygenase (COX) pathway plays a major role in the mediation of the secretory response to exogenous 5-HT in vitro (King et al., 2004). Therefore, indomethacin (10 μM), a COX inhibitor, was routinely applied to the basolateral side of the tissue preparations to abolish the effects of endogenous prostaglandin (Gierse et al., 1995). Treatment with indomethacin resulted in a significant reduction of baseline \( I_{SC} \) in both control rats, from 37.28 ± 6.21 to 17.73 ± 3.61 μA/cm², and WIRS rats, from 56.03 ± 6.01 to 30.95 ± 7.04 μA/cm²; however, the baseline \( I_{SC} \) in WIRS rats was still significantly higher than that in control rats after treatment with indomethacin (P < 0.05, Fig. 1 A1). Indomethacin did not have distinct influence on Rte.

Previous studies have reported that the basolateral application of 5-HT produced a concentration-dependent increase in \( I_{SC} \) with an EC_{50} of 5.4 ± 0.8 μM in the rat distal colon (Budhoo and Kellum, 1994). Thus, 5-HT 10 μM was chosen for the following study. The addition of 5-HT to the basolateral side of the colonic mucosa/submucosa preparations elicited an increase in \( I_{SC} \), whereas the apical application of 5-HT had a negligible effect; these results are similar to our previous findings (Ning et al., 2004; Yang et al., 2008). Therefore, the 5-HT and 5-HT receptor agonists/antagonists were added only to the basolateral side of the preparations in the present study. After pretreatment with indomethacin, the 5-HT-induced \( I_{SC} \) in the colonic mucosa/submucosa tissues was 1321.0 ± 162.5 μA min in the WIRS group, which was much higher than that in the control rats, 740.2 ± 69.2 μA min (n = 11, P < 0.001, Fig. 1 B1, B2).

3.2. Expression of mucosal 5-HT4 receptors, sst2 receptors, and submucosal 5-HT3 receptors after water immersion-restraint stress

To investigate the hypersecretion response to 5-HT in the distal colon of stressed rats, the expression of mucosal 5-HT4 receptors, sst2 receptors, and submucosal 5-HT3 receptors, which are involved in the 5-HT-activated somatostatin-dependent inhibitory pathway, was compared between the control and WIRS rats. Compared to the control rats, the mRNA expression of the mucosal 5-HT4 receptors in the WIRS rats was significantly reduced by 49.5% (n = 6, P < 0.05, Fig. 2 A1), but the protein level had no significant difference (Fig. 2 A2, A3). The mRNA levels of the mucosal sst2 receptor were significantly reduced by 31.9% (n = 4, P < 0.05, Fig. 2 B1), and similarly the protein level was decreased by 37.5% (n = 4, P < 0.05, Fig. 2 B2, B3) in the WIRS rats, comparing to the control rats. For the submucosal 5-HT3 receptors, the mRNA and protein levels in the WIRS rats were significantly reduced by 46.6% (n = 6, P < 0.05, Fig. 2 C1) and 31.8% (n = 6, P < 0.05, Fig. 2 C2, C3), respectively, compared to those of the control rats.

3.3. Effects of the 5-HT3 receptor antagonist MDL72222 on 5-HT-induced \( I_{SC} \) in mucosa/submucosa preparations after water immersion-restraint stress

It has been reported that pretreatment with the 5-HT3 receptor antagonist MDL72222 at 0.01 or 0.1 μM significantly enhances 5-HT-evoked \( I_{SC} \) (Yang et al., 2010). Similar to our previous report, the 5-HT3 receptor antagonist MDL72222 (0.01 μM) significantly increased the 5-HT (10 μM)-induced \( I_{SC} \) in mucosa/submucosa preparations (n = 9, P < 0.01, Fig. 3 A, C) in the normal physiological condition. However, after 2 h of water immersion-restraint stress, MDL72222 (0.01 μM) did not significantly affect the 5-HT (10 μM)-induced \( I_{SC} \) (n = 9, P > 0.05, Fig. 3 B, C).

3.4. Effects of the sst2 receptor antagonist on 5-HT-induced \( I_{SC} \) in mucosa/submucosa preparations after water immersion-restraint stress

Somatostatin is secreted from the submucosal plexus and has been known to decrease basal Cl⁻ secretion and forskolin/carbachol induced Cl⁻ secretion in rat colonic mucosa via the activation of sst2 receptor (McKeen et al., 1995). The sst2 receptor antagonist
CYN154806, when used at 0.1–1 μM, is able to inhibit the somatostatin-induced decrease in \( I_{SC} \) (Holliday et al., 2007). The dosage used in our study was 0.5 μM, as was used in previous reports (Yang et al., 2010). In the present study, the basolateral application of CYN154806 (0.5 μM) significantly increased the 5-HT-evoked \( \Delta I_{SC} \) by 74.0% (\( n = 8 \), \( P < 0.01 \), Fig. 4A) in the mucosa/submucosa preparations of the control rats, and all of the tissue preparations manifested an enhanced \( I_{SC} \) response by 5-HT when pretreated with CYN154806 (Fig. 4B). However, in the WIRS rats, CYN154806 (0.5 μM) did not significantly affect the 5-HT (10 μM)-induced \( \Delta I_{SC} \) (\( n = 8 \), Fig. 4A): although five of

**Fig. 1.** Basic characteristics of electrophysiology and 5-HT-induced ion transport in the distal colon mucosa/submucosa preparations of control and WIRS rats. (A1) Baseline \( I_{SC} \). (A2) Transepithelial resistance. (B1) Representative \( I_{SC} \) recording with arrow indicating the application of 5-HT (10 μM, basolateral side) to the mucosa/submucosa preparations. (B2) Summary of 5-HT (10 μM)-induced \( \Delta I_{SC} \). Columns show the mean ± S.E.M., *\( P < 0.05 \); **\( P < 0.001 \); \( n = 11 \) respectively. 5-HT, 5-Hydroxytryptamine; Indo, indomethacin; WIRS, water-immersion restraint stress; \( I_{SC} \), short-circuit current.

**Fig. 2.** The protein and mRNA expression of sst2, 5-HT5, and 5-HT4 receptors in the distal colon of control and WIRS rats. The mRNA (A1, B1, and C1) and protein (A2, B2, and C2) levels of mucosal 5-HT4 receptors (A; \( n = 6 \)), sst2 receptors (B; \( n = 4 \)) and submucosal 5-HT3 receptors (C; \( n = 6 \)). The western blot bands of 5-HT4 receptors, sst2 receptors and 5-HT3 receptors were shown in figures A3, B3, and C3. The difference in expression levels is reflected by variations in the Y-axes. Columns show the mean ± S.E.M., *\( P < 0.05 \). 5-HT, 5-Hydroxytryptamine; sst2 receptor, somatostatin receptor 2; WIRS, water-immersion restraint stress.
eight mucosa/submucosa preparations manifested an increased $I_{\text{sc}}$ response, two and one of them manifested decreased and unchanged $I_{\text{sc}}$ response, respectively (Fig. 4B).

3.5. 5-HT-induced somatostatin release

To further test whether the colonic submucosal 5-HT$_3$ receptors-mediated somatostatin-dependent secretory inhibitory pathway was suppressed in the WIRS rats, RIA was used to measure the somatostatin release of rat mucosa/submucosa preparations in the water-immersion restraint stress and normal conditions. As shown in Fig. 5, the basal levels of somatostatin in the supernatant from the colonic mucosa/submucosa preparations were low in both the control and the WIRS rats. In the control rats, the incubation of the rat colonic mucosa/submucosa preparations with 5-HT (10 $\mu$M) caused a significant increase in somatostatin release: the concentration in the supernatant changed from the basal value of 8.2±1.2 to 15.2±3.0 pg/mg, which is an increase of 84.9% ($n=6$, $P=0.05$, Fig. 5). Pretreatment with the 5-HT$_3$ receptor antagonist MDL72222 (0.1 $\mu$M) significantly inhibited the 5-HT-induced somatostatin release by 44.6%, from 15.2±3.0 to 8.4±0.9 pg/mg ($n=6$, $P=0.05$). However, in the WIRS rats, 5-HT did not significantly enhance the concentration of somatostatin in the

![Fig. 3. Effects of 5-HT receptor antagonists on 5-HT-induced $I_{\text{sc}}$ responses in the distal colon mucosa/submucosa preparations of control and WIRS rats. The effects of pretreatment with MDL72222 (0.01 $\mu$M) on 5-HT (10 $\mu$M)-induced $I_{\text{sc}}$ in control rats (A1, A2) and WIRS rats (B1, B2). A1 and B1: Representative $I_{\text{sc}}$ recordings; A2 and B2: Summary of the effects of MDL72222 (0.1 $\mu$M) on 5-HT-induced $\Delta I_{\text{sc}}$. Numbers in C indicate the number of tissues demonstrating the variable effect on 5-HT-induced $\Delta I_{\text{sc}}$. “↑” or “↓” in C means MDL72222 enhanced or inhibited 5-HT-induced $\Delta I_{\text{sc}}$ by more than 10%, and “−” means MDL72222 enhanced or inhibited 5-HT-induced $\Delta I_{\text{sc}}$ by less than 10%. Columns show the mean ± S.E.M.; **, $P<0.01$; n = 9 respectively. 5-HT, 5-Hydroxytryptamine; WIRS, water-immersion restraint stress; $I_{\text{sc}}$, short-circuit current.]

![Fig. 4. Effect of the sst$_2$ receptor antagonist on 5-HT-induced $I_{\text{sc}}$ responses in the distal colon mucosa/submucosa preparations of the control and the WIRS rats. (A) Summary of the effect of CYN154806 (0.5 $\mu$M) on 5-HT-induced $\Delta I_{\text{sc}}$. (B) Effect of CYN154806 (0.5 $\mu$M) on 5-HT-induced $\Delta I_{\text{sc}}$. Numbers in the table indicate the number of tissues demonstrating the variable effect on 5-HT-induced $\Delta I_{\text{sc}}$ of CYN154806. “↑” or “↓” in B means CYN154806 increased or decreased 5-HT-induced $\Delta I_{\text{sc}}$ by more than 10%; “−” means CYN154806 increased or decreased 5-HT-induced $\Delta I_{\text{sc}}$ by less than 10%. Values are means ± S.E.M.; **, $P<0.01$; n = 8 respectively. 5-HT, 5-Hydroxytryptamine; sst$_2$ receptor, somatostatin receptor 2; WIRS, water-immersion restraint stress; $I_{\text{sc}}$, short-circuit current.]
Discussion

In disorders such as peptic ulcer, irritable bowel syndrome and Santos et al., 1999; Saunders et al., 2002), chromaffin permeability through the peripheral 5-HT released from the entero-
(Nishioka et al., 1993), and CRH is able to increase ion secretion and/or modifi-
that the colonic submucosal 5-HT3 receptor-mediated somatostatin-
an inhibitory effect on the ion secretion of the rat colon by the tors elicits submucosal somatostatin release, which in turn produces SC responses in the colonic mucosa/submucosa preparations of control and WIRS rats. A summary of the effects of pretreatment with 5-HT3 antagonist, MDL72222 (0.1 μM) on the 5-HT (10 μM)-stimulated somatostatin release. Values are means ± S.E.M., * P<0.05 compared with 5-HT treatment alone in the control; n = 6 respectively. 5-HT, 5-Hydroxytryptamine; WIRS, water-immersion restraint stress.

supernatant, and blocking the 5-HT3 receptors with MDL72222 (0.1 μM) had no significant effect on the 5-HT-induced somatostatin release. Furthermore, the concentration of 5-HT-induced somato-
stantin release was significantly lower in WIRS rats (11.3 ± 2.4 pg/mg) than that in control (15.2 ± 3.0 pg/mg) (n = 6, P<0.05).

4. Discussion

We have previously demonstrated the dual role of the 5-HT3 receptors on electrolyte secretion in the rat distal colon, which includes the stimulatory effect of the 5-HT3 receptors in the myenteric plexus and the inhibitory effect of the 5-HT3 receptor in the submucosal plexus (Yang et al., 2008). The inhibitory effect is somatostatin-dependent. The activation of submucosal 5-HT3 receptors elicits submucosal somatostatin release, which in turn produces an inhibitory effect on the ion secretion of the rat colon by the mediation of sst2 receptor (Yang et al., 2010). Somatostatin activates sst2 receptor either in non-somatostatin submucosal neurons to inhibit the excitability of the secretomotor neurons (Foong et al., 2010) or in colonocytes to inhibit colonic ion secretion (Warhurst et al., 1996).

Physical and psychological stresses are widely accepted as triggers and/or modifiers of the clinical course of diverse gastrointestinal disorders such as peptic ulcer, irritable bowel syndrome and inflammatory bowel disease (Bhatia and Tandon, 2005; Wilhelmsen, 2000). The water-immersion restraint stress is one of the most widely-used experimental restraint stress models; it involves elements of physical stress in addition to psychological stress (Soderholm and Perdue, 2001). It is reported that the release of corticotropin-releasing hormone (CRH) is increased under water-immersion restraint stress (Nishioka et al., 1993), and CRH is able to increase ion secretion and permeability through the peripheral 5-HT released from the entero-


Previous studies have reported that acute restraint stress can significantly increase endogenous 5-HT release and that the released 5-HT accelerates colonic transit via the 5-HT3 receptors (Haga et al., 1995; Hirata et al., 2008a; Nakade et al., 2007). These in vivo studies have shown that 5-HT acts as a mediator of stress-induced colonic dysfunction, and 5-HT3 receptors in the entire colon act as key regulators in stress-induced colonic hypersecretion (Hirata et al., 2008b). However, based on our previous work on the dual role of 5-HT3 receptor, our present study suggests that in acute water immersion-restraint stress, the inhibitory effect of the 5-HT3 receptor in the submucosal plexus is weakened significantly, which may also contribute to the stress-induced colonic hypersecretion.

In summary, 5-HT-induced rat colonic secretion is significantly increased in acute water immersion-restraint stress, and this increase is related to the down-regulation of secretoinhibitory action that is mediated by the submucosal 5-HT3 receptors, mucosal sst2 receptors, and 5-HT-induced somatostatin release. This study contributes to the understanding of the mechanisms underlying the gastrointestinal symptoms and discomfort that are associated with acute stress.

Conflict of interest

The authors have no financial, consultant, institutional and other relationships that might lead to bias or a conflict of interest.

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References


Fig. 5. Radioimmunoassay of somatostatin release in colonic mucosa/submucosa preparations of control and WIRS rats. Values are means ± S.E.M., * P<0.05 compared with 5-HT treatment alone in the control; n = 6 respectively. 5-HT, 5-Hydroxytryptamine; WIRS, water-immersion restraint stress.

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