Luminally released serotonin stimulates colonic motility and accelerates colonic transit in rats

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In this study, we hypothesize that luminally released 5-HT from EC cells of the proximal colon is transferred distally with feces and stimulates motility of the distal colon, resulting in acceleration of colonic transit in rats. We also investigated the mechanism of luminal release of 5-HT of the rat proximal colon.

There is accumulated evidence that a variety of stress stimulates colonic motility, colonic transit, and fecal pellet output (9, 21, 35, 38, 47). Stress-induced acceleration of colonic motor function is mediated via a central CRF pathway (26, 30, 32, 44). In this study, we also investigated whether stress-stimulated colonic motility is mediated via a luminal release of 5-HT.

MATERIALS AND METHODS

Animals. All procedures used in this study were approved by Durham Veterans Affairs Medical Center (Durham, NC). Male Sprague-Dawley rats weighing 250–350 g were used and fed with laboratory rodent chow and water ad libitum.

Measurement of tissue and fecal content of 5-HT. Rats were anesthetized with pentobarbital sodium (45 mg/kg ip) and perfused for 5 min via the left ventricle with 0.01 M PBS at 30 ml/min to wash out the blood. Segments of proximal and distal colon were taken out and cut open along mesentery, and mucosa and submucosa were scraped off by a razor blade. Feces were also removed from the colon. The samples were weighed and homogenized for 2 min in 3 ml of 0.1 N perchloric acid at 4°C, then centrifuged for 30 min at 3,000 rpm at 4°C. The supernatant was filtered with a 0.45 µm centrifuge filter tube (Coster, Corning, NY) for 30 min at 3,000 rpm at 4°C. Samples were diluted to 100 µl with 0.1 N perchloric acid, and then 10 µl of sample were injected into HPLC to measure 5-HT content.

Measurement of luminal concentration of 5-HT. Our preliminary study showed that the 5-HT content in fecal matter was almost the same between the proximal and distal colon. To further investigate the luminal concentration of 5-HT of the proximal and distal colon, we developed the microdialysis assay system of luminal 5-HT in conscious rats.

Rats were anesthetized with pentobarbital sodium (45 mg/kg ip). An indwelling Silastic cannula (ID, 3 mm; OD, 4 mm) was inserted into the proximal or distal colon, and the cannula was run under the skin to an opening made in the back of the neck. 5-HT may leak into the proximal or distal colon, and the cannula was run under the skin to an opening made in the back of the neck. 5-HT may leak into the proximal or distal colon, and the cannula was run under the skin to an opening made in the back of the neck. 5-HT may leak into the proximal or distal colon, and the cannula was run under the skin to an opening made in the back of the neck.
microdialysis probe (A-M-8-4, Eicomp, Japan; OD, 0.2 mm) was inserted into the colonic lumen through the Silastic cannula. A polyethylene (PE) tube (ID, 0.6 mm; OD, 0.9 mm; PE-50) was connected to the distal and proximal end of the microdialysis probe. Three hours after the recovery from the isoflurane anesthesia, saline was infused at 1 μl/min into the microdialysis system. Effluent was collected every 15 min and 5-HT concentration of effluent was measured by HPLC.

Isolated vascularly and luminally perfused colon ex vivo. Rats were fasted overnight and anesthetized with pentobarbital sodium (45 mg/kg ip). The proximal colon, including 0–5 cm from the ileocecal junction was used for isolated vascularly and luminally perfused colon study. As previously described (27), arterial perfusion was achieved through an aortic cannula with the tip lying to the superior mesenteric artery. All vessels apart from those leading into the proximal colon were cut between double ligatures. Those leading into the duodenum and distal colon were only ligated. The stomach, jejunum, ileum, pancreas, and spleen were removed. Vascular perfusion was performed with Krebs solution containing 0.2% BSA, 3% dextran (MW; 35,000–45,000) and 0.25% glucose. The perfusate was saturated with formed with Krebs solution containing 0.2% BSA, 3% dextran (MW; 35,000–45,000) and 0.25% glucose. The perfusate was saturated with 95% O2:5% CO2 to maintain a pH 7.4. The vascular perfusate was collected via a cannula inserted into the portal vein. Luminal perfusate was collected via a cannula inserted into the distal end of the colon. Luminal perfusion was performed with saline. The flow rates for vascular and luminal perfusion were maintained at 3 ml/min and 1 ml/min throughout the experiment, respectively. Both perfusate and the preparation were kept at 37°C throughout the experiment using thermostatically controlled heating apparatus. Samples of both luminal and vascular effluents were collected in ice-cold beaker.

5-HT contents of the luminal and vascular perfusate were measured by HPLC. Ten microliters of 1 M perchloric acid were added for each sample. The mixture was centrifuged at 3,000 rpm at 4°C. The supernatant was filtered with a 0.45 μm centrifuge tube filter (Coster, Corning, NY) for 30 min at 3,000 rpm at 4°C, and then 10 μl samples were injected into the HPLC to measure 5-HT content.

To investigate the effect of an increase of luminal pressure on 5-HT release, after a 30-min equilibration period, samples were collected from both luminal and vascular effluent before (every 15 min × 3 times) and after (every 5 min × 4, then 15 min × 2 times) increase of luminal pressure. Luminal pressure was raised by clamping luminal effluent and clamp was released when pressure reached 10 cmH2O. We used the 10-cmH2O pressure increment, because most of physiological pressure of rat proximal colon is not more than 10 mmHg (8).

To investigate whether luminal pressure-stimulated 5-HT release is mediated via neurons, TTX (3 × 10−7 M) was added in vascular perfusate from 30 min before pressure increase to the end of experiment.

Colonic transit study. To investigate whether intraluminal administration of 5-HT accelerates colonic transit, we performed colonic transit study using a nonabsorbable radioactive marker51Cr. Rats were anesthetized with pentobarbital sodium (45 mg/kg ip), and an indwelling Silastic cannula was inserted into the proximal colon. The cannula was run under the skin to an opening made in the back of the neck, as previously reported (37, 45).

Five to seven days after the operation, colonic transit study was performed. After an overnight fast, 5-HT (10−7–10−5 M, 0.5 ml) or saline (0.5 ml) was administered with51Cr (0.5 μCi; Na51CrO4 in 0.2 ml saline) into the catheter positioned in the proximal colon. After 90 min, rats were killed, and the entire colon was surgically removed and divided into 10 equal segments. The radioactivity of each segment was counted by a gamma counter. The geometric center was calculated using the following equation, as previously reported (13, 37): Geometric center = Σ(fraction of 51Cr per segment × segment number).

Effect of luminal administration of ondansetron on colonic motility. A strain gauge transducer was sutured on the distal colon to record circular muscle contractions. An indwelling Silastic cannula was inserted into the distal colon to administer ondansetron (5-HT3 receptor antagonists) or saline. Wires from a strain gauge transducer and cannula were put through a subcutaneous tunnel and out the dorsum. Five days after the operation, the colonic motility study was performed.

Rats were placed in a cage, and wires from a strain gauge transducer were connected to the recording system (Power Lab/4SP; AD Instruments, Colorado Springs, CO). After a basal recording for 120 min, rats were loaded with a restraint stress, as previously reported (5, 30). In this restraint stress, the animal was able to move its limbs and head but not its trunk (5, 30).

To investigate the effect of luminal administration of ondansetron, on stress-induced stimulation of motility of distal colon, ondansetron (10−6 M, 0.5 ml) or saline (0.5 ml) was administered into the lumen of the distal colon via a cannula 60 min after the start of stress loading.

The area under the curve of the motility recording was measured as a motility index (MI) by using a computer-assisted system (Power Lab) as previously reported (45). Calculated MI before administration of ondansetron or saline for 30 min was expressed as 100% (control), and the MI after administration of ondansetron or saline for 30 min was expressed as a percentage of the control.

Materials. BSA, dextran, glucose, 5-HT, and TTX were purchased from Sigma (St. Louis, MO).51Cr was purchased from Amersham (Arlington, IL).

Statistical analysis. Results were expressed as means ± SE. The data were evaluated by Student’s t-test, paired t-test, or repeated-measures ANOVA followed by Dunnett’s test. Differences were considered statistically significant at P < 0.05.

RESULTS

Tissue content of 5-HT in the proximal colon (15.2 ± 4.3 ng/mg wet tissue, n = 5) was significantly higher than that in the distal colon (3.3 ± 0.7 ng/mg wet tissue, n = 5, P < 0.01) (Table 1).

In contrast, 5-HT content in fecal matter was almost the same in the proximal (790 ± 168 pg/mg wet feces, n = 6) and distal colon (796 ± 146 pg/mg wet feces, n = 6) (Table 1). 5-HT concentrations of effluent detected by microdialysis were almost the same in the proximal colon (9.47 ± 1.00 pg/μl, n = 5) and distal colon (10.22 ± 2.25 pg/μl, n = 5) (Table 1). Elevation of intraluminal pressure (10 cmH2O) increased the luminal content of 5-HT (883 ± 50% increase of basal, n = 5, P < 0.05). In contrast, no significant increase of 5-HT

<table>
<thead>
<tr>
<th>Tissue Content, ng/mg wet wt</th>
<th>Fecal Content, pg/mg wet wt</th>
<th>Luminal Content, pg/μl Effluent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proximal colon</td>
<td>15.2 ± 4.3 (n=5)</td>
<td>790 ± 168 (n=6)</td>
</tr>
<tr>
<td>Distal colon</td>
<td>3.3 ± 0.7 (n=5)*</td>
<td>796 ± 146 (n=6)</td>
</tr>
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Table 1. Tissue content, fecal content, and luminal content of 5-HT in the proximal and distal colon

Tissue content of 5-HT in the distal colon was significantly lower than that in the proximal colon (*P < 0.01). In contrast, 5-HT content in fecal matter was almost the same between the proximal and distal colon. Similarly, luminal content of 5-HT detected by microdialysis was almost the same between the proximal and distal colon.
content was observed in the vascular perfusate (113 ± 11% increase of basal, n = 5) (Fig. 1A).

Pretreatment with TTX significantly reduced the increase of luminal 5-HT content in response to luminal pressure increment (Fig. 1B). Geometric center of colonic transit was 5.14 ± 0.43 in saline-treated rats (n = 7). Luminal administration of 5-HT (10⁻⁶–10⁻⁵ M) significantly increased geometric center (Fig. 2).

We have recently shown that intraluminal administration of ondansetron had no effects on basal activity and that restraint stress significantly augmented colonic motility in conscious rats (40). Current study also showed that restraint stress significantly increased the motor activity of the distal colon. Stimulated distal colonic motility in response to restraint stress was attenuated by the luminal administration of ondansetron (Fig. 3).

DISCUSSION

The largest store of 5-HT in the body is found in GI tract, corresponding to over 95% of the body’s 5-HT. Most of the 5-HT in the GI tract is present in EC cells of the mucosal epithelium. 5-HT released from EC cells in response to chemical or mechanical stimuli affects GI motility (12, 14). 5-HT initiates peristaltic reflexes by acting on intrinsic (16, 19), as well as extrinsic, sensory neurons (13).

5-HT is also present in neurons of the enteric nervous system and acts as the neurotransmitter of a subset of myenteric interneurons (46). However, it remains unclear which 5-HT released from the enteric nervous system or EC cells plays a major role in mediating colonic motility. 5-HT is released from EC cells into the portal circulation or basolateral border of the mucosa (14), while others showed that 5-HT is released into the lumen of GI tract (1, 12). Immunoelectron microscopic study revealed that aggregation of secretory granules of 5-HT was located in the apical and basolateral cytoplasm of EC cells in the rat duodenum. After the increase of intraluminal pressure, many apical secretory granules were no longer dense, and particles were localized over the cytoplasmic matrix and microvilli (12). These findings indicate that 5-HT is stored in the secretory granules of EC cells and released into the cytoplasmic matrix and then diffuses or is transported into the intestinal lumen in response to intraluminal pressure increase.

Electrical stimulation of the vagus nerves or duodenal acidification evokes 5-HT release from EC cells into the intestinal lumen in concentrations as high as 1.9 μM (25, 28, 49). There is also evidence that central vagal nerve stimulation stimulates luminal release of 5-HT in the rat stomach (48).

Over 20 years ago, Cooke et al. (7) suggested that luminal release of 5-HT from EC cells of the intestinal mucosa is a significant physiological event (7). Luminally applied 5-HT

![Fig. 1. 5-HT release from the vascularly perfused rat proximal colon. A: intraluminal pressure increase (10 cm H₂O) was applied during 45–50 min. Intraluminal pressure increase significantly increased the 5-HT content of the luminal perfusate but not the vascular perfusate. Elevation of intraluminal pressure increased the luminal content of 5-HT (883 ± 290% increase of basal, n = 5, P < 0.05 compared with basal) but not the vascular content 5-HT (113 ± 11% increase of basal, n = 5). B: effect of elevation of intraluminal pressure on luminal release of 5-HT was significantly reduced by TTX (140 ± 18% increase of basal, n = 5). Percent increase of basal release is calculated as value of 5-HT release/value of 15 min × 100.](http://ajpregu.physiology.org/)

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can move by passive diffusion across the intestinal wall of the guinea pig ileum (7). A recent study also showed that 5-HT can cross the intestinal wall from the mucosa to the serosa (apical-to-basolateral direction) (34). Thus, 5-HT into the intestinal lumen could reach the synaptic circuitry resulting in stimulation of 5-HT receptors located on the lamina propria and/or enteric nervous system. We have previously showed that luminaly released 5-HT from EC cells stimulates 5-HT3 receptors located on the vagal sensory fibers. The sensory information is transferred to the vagal efferent and stimulates the release of ACh from the colonic myenteric plexus, resulting in muscle contraction (13).

Our current study demonstrated that 5-HT is released into the lumen but not into the portal circulation in response to luminal pressure increase of the rat proximal colon. It is likely that the basolateral secretion of 5-HT leaks to the luminal side of gut wall. However, our results showed that luminal pressure increase causes only luminal concentration of 5-HT, but not vascular concentration of 5-HT. This suggests that luminal pressure increase stimulates EC cells to release 5-HT into the colonic lumen.

5-HT activates enteric afferent neurons to stimulate intestinal motor function (4, 11, 23). We have previously showed that luminal administration of 5-HT (10⁻⁶–10⁻⁵ M) into the proximal colon significantly increased the fecal pellet output in rats (13). Our current study also demonstrated that luminal administration of 5-HT (10⁻⁶–10⁻⁵ M) into the proximal colon significantly accelerated colonic transit. This suggests that luminal 5-HT is involved in mediating colonic transit and motility.

It has been shown that the number of EC cells is significantly higher in the proximal colon, compared with that of the distal colon (41). Our current study also showed that mucosal content of 5-HT was significantly higher in the proximal colon than than that of the distal colon. However, the physiological role of abundant number of EC cells and 5-HT content in the proximal colon remain unknown.

As the concentration of 5-HT in wet feces, rather than dry feces, may reflect the physiological concentration in the co-

Fig. 2. Effect of intraluminal administration of 5-HT on the colonic transit. Geometric center of colonic transit was 5.14 ± 0.43 in saline-treated rats (n = 7). Administration of 5-HT (10⁻⁶ M) and 5-HT (10⁻⁵ M) significantly increased geometric center to 6.87 ± 0.58 (n = 6) and 6.95 ± 0.45 (n = 5), respectively. Thus, luminal administration of 5-HT (10⁻⁶–10⁻⁵ M) significantly accelerated colonic transit (*P < 0.05).

Fig. 3. Effect of luminal administration of ondansetron on stress-induced stimulation of colonic motility (A) and MI change (B). Ondansetron significantly reduced stress-induced stimulation of colonic motility. Calculated MI before administration of ondansetron or saline for 30 min was expressed as 100%, and the MI after the administration of ondansetron or saline for 30 min was expressed as a percent MI change. Ondansetron (62.8 ± 7.2%, n = 4) significantly reduced MI change compared with saline (96.0 ± 5.2%, n = 4) (*P < 0.05) (B).
Ionic lumen, 5-HT content in feces was shown as wet weight of feces. We demonstrated that the 5-HT content in feces was almost the same between the proximal and distal colon. Microdialysis study confirmed that there was no significant difference of luminal content of 5-HT between the proximal and distal colon in conscious rats.

In the central and peripheral nervous system, 5-HT is primarily inactivated by reuptake into the 5-HT neurons that secrete it. This reuptake is mediated by a highly selective plasma membrane 5-HT transporter (SERT). Mucosal epithelial cells also express SERT in the basolateral and apical cell membranes of the GI tract. Thus, mucosal epithelial cells take up 5-HT to inactivate it (33, 34). However, it is unlikely that SERT is present in the colonic lumen. This raises the possibility that 5-HT released from EC cells of the proximal colon into the lumen may be transferred to the distal colon with feces. It is conceivable that luminal 5-HT released from the proximal colon, in addition to 5-HT released from the distal colon, may play an important role in mediating the distal colonic motility.

Restraint stress is well known to stimulate colonic motility via stimulating CRF and autonomic nervous system in rats (6, 29, 44). In addition, endogenous 5-HT seems to be involved in stress-induced stimulation of colonic motility. Stress-induced simulation of colonic motility is antagonized by a systemic treatment with 5-HT3 receptor antagonists (36). We have recently showed that restraint stress augmented colonic motility and increased the luminal release of 5-HT of the proximal colon in rats (40). Thus, luminally released 5-HT has an important role in stress-induced stimulation of colonic motility.

Our current study demonstrated that restraint stress -induced stimulation of motility of the distal colon was abolished by intraluminal administration of ondansetron into the distal colon. This suggests that 5-HT is released into the colonic lumen in response to restraint stress and that released 5-HT stimulates colonic motility via 5-HT3 receptors.

Not only 5-HT3 receptors but also 5-HT4 receptors mediate colonic motility and fecal pellet output in response to endogenous or exogenous 5-HT in rodents (2, 18, 22, 24, 39, 42, 43). In mouse, rat, and human intestine, the peristaltic reflex is initiated by mucosal release of 5-HT and activation of 5-HT4 receptors on CGRP sensory neurons, which is relayed to VIP/nitric oxide inhibitory motor neurons and to ACh/tachykinin excitatory motor neurons (17) Further studies are needed to address whether 5-HT4 receptors are involved in mediating the stimulation of colonic motility in response to restraint stress in rats.

The mechanism of 5-HT release into the lumen in response to luminal pressure increase remains to be investigated. Mechano-sensitive afferent fibers exist with their ending in the serosa, muscle, and mucosa of rat colon in vitro (31). It is suggested that EC cells are stimulated by increase of luminal pressure to release 5-HT via mechano-sensitive neural pathways of intramural nerve plexus. Recent study showed that 5-HT release evoked by muscle stretch is abolished by the pretreatment with TTX in guinea pig ileum (3).

In contrast, others showed that the stimulatory effect of luminal release of 5-HT by high luminal pressure (25–35 cmH2O) was not altered by TTX of the rat duodenum (12). In their study, they used the rat duodenum and intraluminal pressure was raised to 25–35 cmH2O. In our study, we used the rat proximal colon. Pressure was raised to 10 cmH2O because the majority of the wave amplitude of normal peristaltic in the proximal colon is less than 10 mmHg (7.6 cmH2O) in rats (8). The mechanism of luminal release of 5-HT may depend on the degree of luminal pressure and/or organs.

We demonstrated that TTX significantly reduced 5-HT release induced by intraluminal pressure increase (from 883 ± 290% to 140 ± 18% increase of basal) (Fig. 1B). This suggests that 5-HT release in response to luminal pressure increase is mainly regulated via neural mechanisms. However, we cannot exclude the possibility that luminal pressure increase directly stimulates release of 5-HT from EC cells, because TTX could not completely abolish 5-HT release in response to luminal pressure increase.

In conclusion, 5-HT is released intraluminally in response to an increase of luminal pressure and restraint stress of the rat proximal colon. It is likely that released 5-HT is carried to the distal colon with feces to stimulate colonic motility and transit. Thus, 5-HT released into the colonic lumen may play an important role in mediating colonic peristalsis and stress-induced stimulation of colonic motility.

GRANTS

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REFERENCES


