Colorectal Distention and Intraluminal Cholera Toxin Induce Propulsive Contractions Through 5-HT₃ Receptors on Pelvic-nerve Afferents in Decerebrated Rats

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ABSTRACT. It is well known that 5-hydroxytriptamine-3 (5-HT₃) receptor antagonists prevent nausea and vomiting caused by the release of 5-HT from enterochromaffin cells in patients treated with chemical or radiation therapy for cancer, and that these antagonists produce constipation. Consistently, 5-HT₃ receptor agonists have been reported to alleviate constipation. These antagonists prevent the binding of 5-HT to receptors on abdominal vagal afferents, and thus produce an antiemetic effect. These findings suggest that the defecation reflex may be induced by 5-HT released from enterochromaffin cells. This study was planned to examine this possibility in decerebrate rats. Since intestinal distention and intraluminal cholera toxin have been reported to release 5-HT from the intestinal wall, we used both stimuli to stimulate enterochromaffin cells. Intraluminal infusion of cholera toxin (0.2 mg in 1 ml saline) enhanced colorectal propulsive contractions, and this enhancement was significantly attenuated by i.v. application of the 5-HT₃ receptor antagonist granisetron, and also by bilateral severance of the pelvic nerve. Colorectal contractions in response to colorectal distension were also significantly inhibited by both treatments. These results are consistent with the assumption noted above and suggest that cholera toxin and colorectal distension induce the defecation reflex through the activation of colorectal pelvic-nerve afferents via 5-HT₃ receptors and 5-TH released from enterochromaffin cells.

Key words : enterochromaffin cell — 5-TH — 5-TH₃ receptors — defecation reflex — granisetron

5-hydroxytriptamine-3 (5-TH₃) receptor antagonists, e.g., dolasetron, granisetron, ondansetron and tropisetron, are well known to relieve acute nausea and vomiting associated with chemical and radiation cancer therapies.[1] Based on several findings, the main site of the antiemetic action of 5-TH₃ antagonists is now thought to be 5-TH₃ receptors on peripheral terminals of abdominal vagal afferents : 1) Cancer chemotherapy causes the release of 5-HT from enterochromaffin cells in the intestinal mucosa.[2-5] 2) 5-HT acts on 5-HT₃ receptors at peripheral ends of vagal afferents and

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produces afferent activities.[6]   3) Activities of abdominal vagal afferents induce prodromal signs and vomiting.[7]   4) 5-HT$_3$ receptor antagonists prevent these vagal afferent activities and emetic responses.[8,9]

On the other hand, 5-HT receptor antagonists are also known to cause moderate constipation as a side effect.[10]   Consistent with this trend, a novel 5-HT$_3$ receptor agonist, YM-31636, has been shown to facilitate defecation in ferrets.[11,12]   The defecation reflex is well known to be induced by pelvic nerve afferent activity.[13-15]

These findings suggest that 5-HT$_3$ receptors on the peripheral terminal of pelvic-nerve afferents may participate in induction of the defecation reflex, similar to the induction of emesis by vagal afferents.   We performed this study to examine this possibility using cholera toxin[16-20] and colonic distention,[21,22] which have been reported to release 5-HT from enterochromaffin cells.

MATERIALS AND METHODS

1. Animal preparation : Adult male Sprague-Dawley rats, each weighing 280-350 g, were obtained from Japan Clea (Hyogo, Japan).   They were housed in a climate-controlled, 12 hr light/12 hr dark room, and received water and food *ab libitum*.   They were deprived of food for 24 hr before the experiment, but had free access to water.   The Animal Research Committee of Kawasaki Medical School approved this experimental protocol.

Nagano *et al* (2004) recently demonstrated that colorectal propulsive contractions in response to colorectal distension and anal canal stimulation are mediated by the pontine defecation reflex center in rats.[23]   The supaspinal reflex pathway is sensitive to anesthesia as shown by Maggi *et al* (1988).[24]   Therefore we performed this study in decerebrated rats.   The rats were anesthetized with an injection of alpha-chloralose (60 mg/kg) into the tail vein.   When the animals became flaccid at about 10 min after the injection, pentobarbital sodium (16 mg/kg) was injection into the tail vein to achieve surgical anesthesia.   A trachea cannula was inserted to maintain the respiratory tract, and the head was fixed in a head-holder.   The dorsolateral surface of the skull was exposed through an incision along the mid line, and the temporal muscle was stripped off to expose the parietal and temporal bones.   Both bones and the underlying dura mater were bilaterally removed to expose the cerebral hemispheres.   A slender knife (2 mm wide), specially made from a razor blade, was inserted through the right and then left hemispheres at the level of the lambdoid suture, and the midbrain was severed at the rostral part.   Body temperature was maintained near 37°C with a heating pad.   The experiments were started about 4 hr later, after the rats had recovered from the anesthetic and the corneal reflex could be observed.

2. Recording intraluminal pressure and perfusion of the colonic lumen : Intraluminal pressure was simultaneously recorded from the descending colon and rectum by a balloon-pressure transducer method.   Balloons (15 mm in length) were made from the head of a condom and placed on the end of a vinyl tube.   The colonic and rectal balloons were arranged at 25-mm

intervals using spacers of 3 lengths of stainless steel wire. The vinyl tube of the colonic balloon was bundled with another tube, which was used for the manual perfusion of saline and cholera toxin (0.2 mg in 1 ml of saline) with a syringe into the colonic lumen between both balloons. The vinyl tube of the rectal balloon was also bundled with a polyethylene tube (4 mm in outer diameter). The polyethylene tube allowed passive outflow of these perfused solutions.

An electrode assembly was used to stimulate the anal-canal mucosa. To deliver stimulating current over the mucosa, the assembly consisted of three pairs of platinum-wire electrodes (0.3 mm in diameter and 8 mm long) arranged on the outer surface of a piece of vinyl tube (1 cm in length and 5 mm in outer diameter). The anal mucosa was usually stimulated with pulses of 10 V, 20 Hz, and 0.5-ms duration. The vinyl tube of the rectal balloon and the polyethylene tube were passed through the vinyl tube of the electrode assembly and fixed as shown in Fig 1. The descending colon was ligated at the most oral part and a wound was made on the wall just anal to the ligation. The balloon array was inserted through the anal canal to the rectum and descending colon until the electrode assembly fit into the anal canal, while the tubes for the colonic balloon and infusion were led out through the wound on the colonic wall. To record intraluminal pressure, each balloon was connected to a pressure transducer and a 1-ml syringe via a T-cock. Using the syringe, the colonic and rectal balloons were filled with 0.2 and 0.1 ml of air, respectively. The balloons were also used to induce the defecation reflex by further distension with an additional injection of 0.2 or 0.3 ml of air. The pelvic nerves were isolated at the part just peripheral to the bifurcation of the pudendal nerve in both sides. The isolated part of the nerve was loosely tied with thin thread. After control recording of colorectal contractions was obtained, the thread was pulled out to rip down the pelvic nerve. Arterial pressure was monitored from the femoral artery through a thin polyethylene cannula filled with heparinized Tyrode's solution.

To quantify colorectal contractions, we measured the amplitudes and half-amplitude durations [(a) and (b), respectively, in Fig 2A] for all contractions produced during the 15-min periods before and after an experimental procedure. We also calculated the sum of (a) by (b) for each contraction in these 15-min periods and used this value as a motility index. The differences between motility indices were statistically evaluated using Student's t-test (paired). Probabilities of less than 0.05 were considered statistically significant.

RESULTS

1. Effects of cholera toxin and granisetron on colorectal motility: Figure 2A shows a typical result of experiments performed to observe the effects of cholera toxin on colorectal contractility. Cholera toxin (0.2 mg in 1 ml of saline) was manually infused into the colonic lumen between the colonic and rectal balloons (see Fig 1). Although the infusion was performed slowly over about 2 min (horizontal bar at the bottom of Fig 2A) and the perfusate was allowed to freely flow out through the drain tube (see Fig 1),
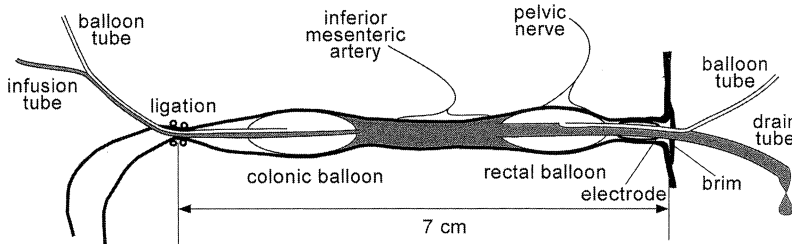
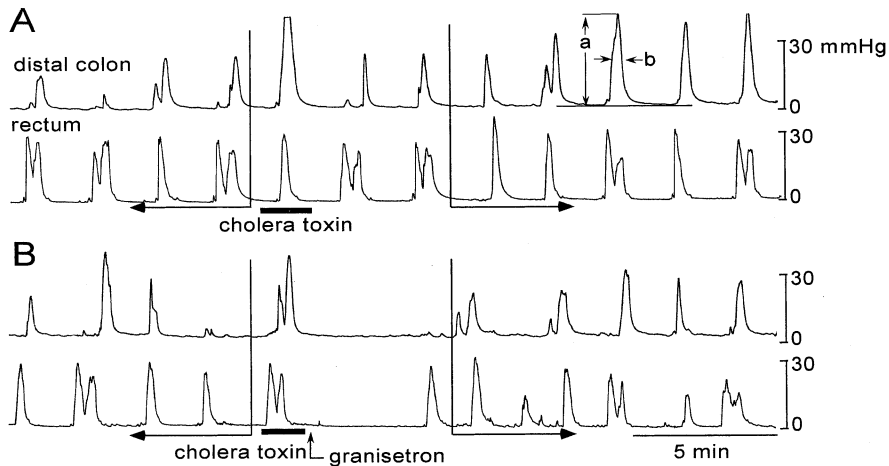Fig 1. Schematic representation of the experimental set-up

Fig 2. Effects of cholera toxin and granisetron on colorectal contractility. A : Effects of cholera toxin. Cholera toxin (0.2 mg in 1 ml saline) was infused during the period indicated by the horizontal bar. B : Effects of sequential application of cholera toxin and granisetron. The vertical line and horizontal arrow indicate periods in which the motility index was measured.

the infusion itself enhanced each colonic and rectal contraction in this case. After that, colorectal contractions became similar to those seen before the infusion, and these continued for about 8 min. These contractions were then gradually augmented in amplitude. This augmentation was reproducibly produced in the 5 other rats and was sustained during the observation period for about 30 min after infusion. Thus, we decided that this augmentation was an effect of cholera toxin. Therefore, we measured colorectal motility indices during the 15-min period from about 8 min after the end of infusion. Over the subsequent 15 min, cholera toxin was washed out 3 or 4 times by the slow infusion of 1 ml of saline. For the next 20 minutes, rats were allowed to rest and records of colorectal contractions were obtained. One milliliter of cholera toxin solution was then again infused as described above. Soon after infusion was complete, granisetron (1 mg/kg) was injected into the femoral vein. As shown in Fig 2B, granisetron suppressed colorectal contractions for about 5 min. However, the suppression period did not overlap the augmenting effect of cholera toxin. Therefore, we again measured motility indices during the 15-min period from about 8 min after the end of the infusion of cholera toxin.
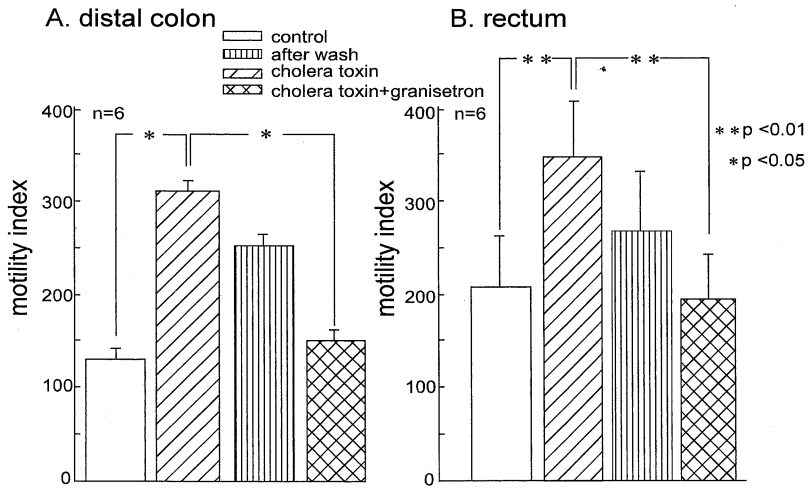
A. distal colon   B. rectum

control
after wash
cholera toxin
cholera toxin+granisetron

**p <0.01
*p <0.05

Fig 3. Quantitative evelution of the effects of cholera toxin and granisetron on colorectal contractions. White columns represent motility indexes (means in 6 rats) of colorectal contractions produced before an intraluminal infusion of cholera toxin. Hatched columns represent motility indexes measured during the 15-min period from about 8 min after the end of the 1st infusion of cholera toxin (0.2 mg in 1 ml of saline). Vertical-striped columns represent motility indexes measured during the 15-min period after the 15-min period in which cholera toxin was washed out 3 or 4 times with an infusion of 1 ml of saline. Checked columns represent motility indexes measured during the 15-min period from about 8 min after the end of the 2nd infusion of cholera toxin, while granisetron (1 mg/kg) was intravenously applied soon after the end of the 2nd infusion.

A. distal colon   B. rectum

*p<0.05
control
cholera toxin

before after
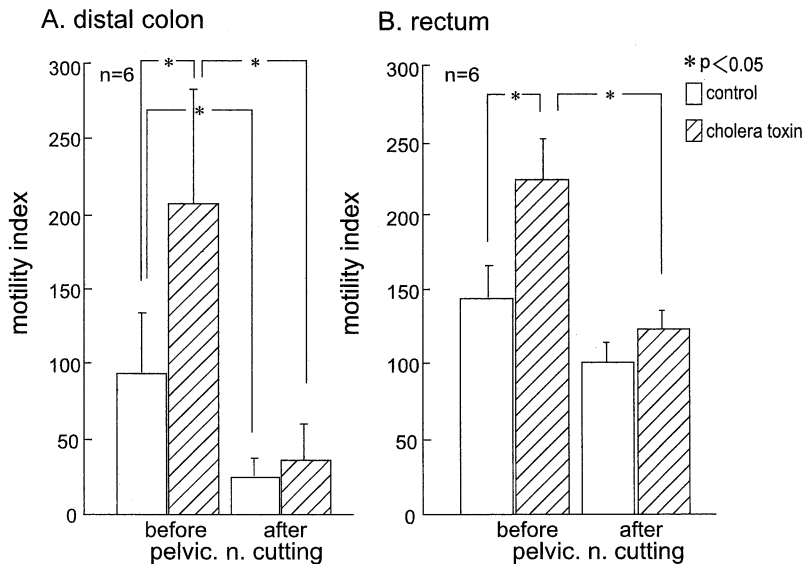pelvic. n. cutting

before after
pelvic. n. cutting

Fig 4. Effects of cutting the pelvic nerves on the augmentation of colorectal contractility by cholera toxin. Paired white and hatched columns represent motility indexes before and after the infusion of cholera toxin (0.2 mg in 1 ml saline), respectively. Therefore, the difference between the columns represents the argumentation by cholera toxin. The augmentations observed before cutting of the pelvic nerves were compared to those obtained after cutting.

Figure 3 shows the means and standard errors of the motility indices obtained from 6 rats.   Cholera toxin significantly increased the colonic motilithy index from 134.0 ± 12.2 to 315.4 ± 13.2, and the rectal-motility index from 176.3 ± 72 to 345.3 ± 72.4.   About half of the augmentation remained after cholera toxin was washed out.   However, after sequential applications of cholera toxin and granisetron, the remaining augmentations almost disappeared and colorectal motility indices returned to near the control levels.   Thus, granisetron might have completely suppressed the augmentative effects of the 2nd administration of cholera toxin.

2. Effects of cutting the pelvic nerves on the augmentation of colorectal contractions by cholera toxin : To evaluate the participation of the defecation reflex in the augmentative effects of cholera toxin on colorectal contractions, the effects of bilateral cutting of the pelvic nerves were observed in 6 rats. The motility index of spontaneous colorectal contractions decreased after cutting of the pelvic nerves (Fig 4), while only the change in colonic motility was significant.   Before cutting of the pelvic nerves, cholera toxin also significantly increased colorectal motility in the 6 rats in this group. The augmentative effects of cholera toxin almost disappeared after pelvic-nerve cutting.
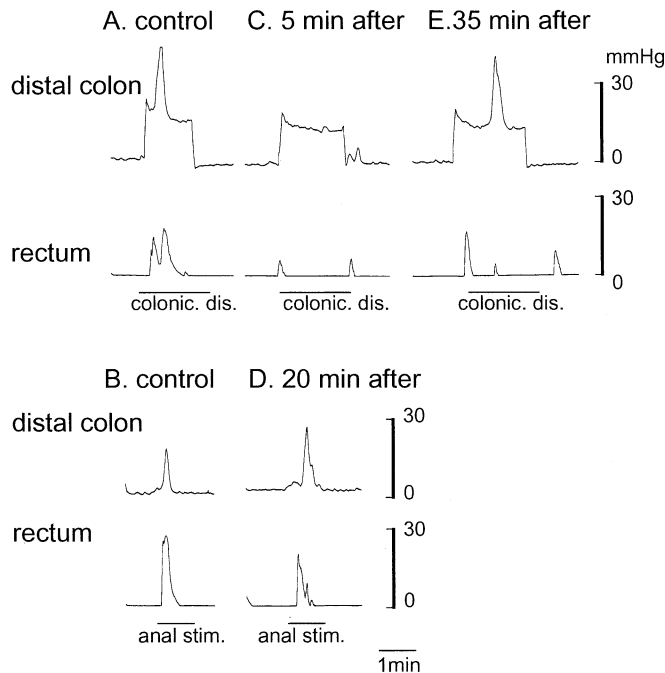
A. control   C. 5 min after   E.35 min after

distal colon

rectum

colonic. dis.   colonic. dis.   colonic. dis.

B. control   D. 20 min after

distal colon

rectum

anal stim.   anal stim.

1min

Fig 5.   Effects of granisetron on colorectal contractions induced by colonic distension and electrical stimulation of the anal mucosa.   A : Control responses of the distal colon and rectum to colonic distension.   B : Control responses of distal colon and rectum to anal stimulation.   Colorectal contractions in response to colonic distention markedly decreased at 5 min after an intravenous injection of granisetron (1 mg/kg) (C), but almost recovered at 35 min (E), while the responses to anal stimulation did not change dramatically at 20 min after granisetron (D).

3. Effects of granisetron and pelvic-nerve cutting on colorectal contractions induced by colonic distension and electrical stimulation of the anal mucosa:
As shows in Fig 5A and C, colorectal contractions in response to colonic distension were markedly attenuated after an i.v. application of granisetron. Consistent with this result, the motility index of the reflex contractions measured in the 6 rats in this group was significantly reduced from 11.6 ± 2.8 to 1.0 ± 0.8 in the colon and from 76.3 ± 3.8 to 27.4 ± 3.1 in the rectum
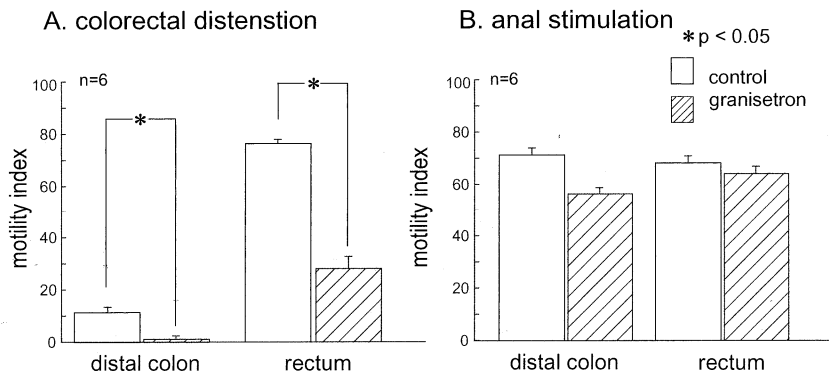

Fig 6. Quantitative evaluation of the effects of granisetron on colorectal contractions induced by colonic distension and electrical stimulation of the anal mucosa. White columns represent motility indexes (means in 6 rats) of colorectal contractions produced during colonic distension (A) or anal-canal stimulation (B) before an intravenous injection of granisetron (1 mg/kg) in 6 rats. Hatched columns represent those induced from 5-20 min after grasisetron.
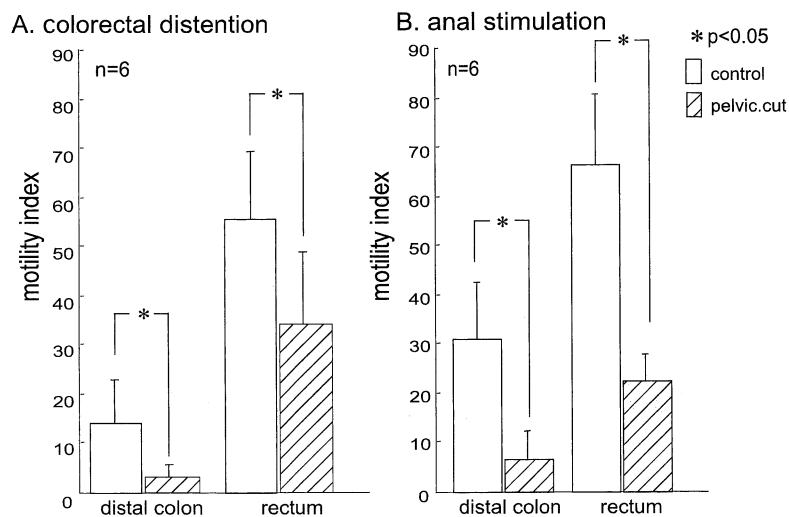

Fig 7. Quantitative evaluation of the effects of cutting the pelvic nerves on colorectal contractions induced by colonic distension (A) and electrical stimulation of the anal mucosa (B). White columns represent motility indexes (means in 6 rats) of colorectal contractions produced during colonic distension (A) or anal-canal stimulation (B) before cutting of the pelvic nerves. Hatched columns represent those induced after cutting.

(Fig 6A).   In contrast, granisetron did not significantly reduce colorectal contractions in response to anal stimulation, as shown in Figs 5B and D and 6B.

Cutting of the pelvic nerves significantly attenuated bowel contractions in reflex responses to colonic distention from 14.4 ± 10.2 to 3.1 ± 2.6 in the descending colon and from 56.3 ± 13.5 to 34.3 ± 15.1 in the rectum (Fig 7A). Similarly, colorectal contractions in reflex responses to anal-canal stimulation significantly reduced from 31.2 ± 11.5 to 7.1 ± 5.7 in the colon and from 66.8 ± 14.0 to 22.6 ± 5.6 in the rectum after cutting of the pelvic nerve (Fig 7B).

DISCUSSION

We obtained the following results in decerebrate rats : 1) Cholera toxin augmented colorectal contractions.   2) Granisetron antagonized this augmentation, and also reduced contractions in reflex responses to colorectal distensions, but did not attenuate contractions in reflex responses to anal-canal stimulation.   3) Cutting of the pelvic nerves decreased spontaneous contractions as well as those in response to cholera toxin, colonic distention and anal-canal stimulation.   To the best of our knowledge, these results are novel, although similar effects of pelvic-nerve cutting on the reflex responses to colorectal distension and anal-canal stimulation have been observed in dogs.[15]

5-HT is produced, stored, and secreted in large amounts by enterochromaffin cells in the gastrointestinal epithelium.[25]   Similar to intraluminal cholera toxin,[16-20] mechanical stimulation of the bowel mucosa[26] and intestinal distention [21,22] are known to release 5-HT from enterochromaffin cells to the intestinal lumen and the portal vein.   5-HT is also known to act as a paracrine mediator and to stimulate vagal afferents via 5-HT_3 receptor (see the Introduction) and enteric sensory neurons comprising the peristaltic reflex arc via 5-HT_{1p} and 5-HT_4 receptors in human jejunum and rat colon, and via 5-HT_3 and 5-HT_4 in guinea pig colon.[26-28]   Colorectal propulsive contractions are known to be elicited through the peristaltic reflex arc consisting of enteric neurons[25] and to be facilitated through the defecation reflex arc composed of pelvic-nerve afferent and efferent neurons,[13-15] and the sacral[13,14] and pontine defecation reflex centers.[15,31,32]

In this study, the intravenous application of a 5-HT_3 receptor antagonist, granisetron, and cutting of the pelvic nerves suppressed most of the augmentative effects of cholera toxin and colorectal distention.   As mentioned above, it has been well established that the peristaltic reflex is induced by 5-HT via 5-HT_{1p} and 5-HT_4 receptors in rats.   Therefore, we assume from these results that 5-HT released from enterochromaffin cells produced most of these augmentative effects via 5-HT_3 receptors on pelvic-nerve afferents and the defecation reflex arc in our preparation of decerebrate rats.   This assumption may be consistent with the previous reports mentioned above.   Further, this assumption is supported by the present result that granisetron, but not cutting of pelvic-nerve afferents, did not suppress the augmentative effects of electrical stimulation of the anal canal, since stimulation of the anal canal may directly activate pudendal-

nerve afferents and elicit the defecation reflex, whereas cutting of the pelvic-nerves severs the efferent limb of the defecation reflex arc.

These results suggest that 5-HT released from enterochromaffin cells activates the defecation reflex via 5-HT$_3$ receptors on pelvic nerve afferents. However, the release of 5-HT from enterochromaffin cells is known to be under facilitative auto-regulation via 5-HT$_3$ receptors, which are sensitive to granisetron.[31-33] Therefore, it seems possible that granisetron decreased 5-HT release in response to cholera toxin and bowel distention in this study. The reduction of 5-HT release should attenuate colorectal contractile responses. Further studies are needed to evaluate this possibility.

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