Integrative control of rectoanal reflex in guinea pigs through lumbar colonic nerves

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METHODS AND MATERIALS

Experimental procedures followed the guidelines of the local animal ethics committee. Experiments were performed on 50 male guinea pigs (315–450 g body wt) anesthetized with ethyl carbamate (0.7–1.0 g/kg ip), artificially ventilated via a trachea cannula, and immobilized with gallamine (0.1 mg/kg iv). The level of anesthesia was intermittently tested after the immobilization was stopped. The postganglionic axons from the inferior mesenteric ganglia travel to the colon, the rectum, and the IAS via the LCNs and hypogastric nerves (HGNs) (2, 10–12). These nerves were viewed with a binocular stereomicroscope and cut extraperitoneally.

Figure 1 shows the protocols used in the present study. Protocols 1 and 2 were performed to analyze the extrinsic rectoanal reflex. Lumbar sympathectomy (LS), composed of section of the LCNs and section of HGNs, was performed with simultaneous section of the intermesenteric nerve fibers to prevent the influence of the superior mesenteric ganglia (8), although the majority of inferior mesenteric ganglion cells project into the LCNs (and HGNs) (10). After laminec- tomy, spinal transection at the 13th thoracic cord (TH 13) was performed with a blunt knife to exclude influence from the supraspinal pathway. Pithing the first to the third sacral cords [PITH (S1–3)] was performed by inserting the needle...
into the vertebral canal to exclude the center of the extrinsic excitatory reflex through the pelvic nerves, leaving behind the intrinsic (enteric) neural pathway. Hemostasis was obtained by inserting cotton wool into the vertebral canal. The difference between protocols 1 and 2 was only in the order of LS and TH 13.

**Protocol 3** was performed to analyze the intrinsic rectoanal reflex. LS and PITH (S1–3) were performed at first to exclude the extrinsic reflexes, and guanethidine sulfate (3 mg/kg iv) was administered to block sympathetic adrenergic nerve terminals, leaving only the intrinsic reflex pathways. To evaluate the contribution of enteric cholinergic and nitrergic nerve pathways to the intrinsic reflex, a nitric oxide (NO) synthase inhibitor, Nω-nitro-l-arginine methyl ester hydrochloride (L-NAME; 10 mg/kg iv); Nω-nitro-l-arginine (L-NNA; 10 mg/kg iv); tetrodotoxin (2 μg/kg iv); L-arginine (L-ARG; 50 mg/kg iv); or atropine sulfate (0.5 mg/kg iv) was administered. Apart from protocol 3, we investigated the effect of atropine sulfate (0.5 mg/kg iv) on the reflex-mediated rectal contraction and that of L-NAME (10 mg/kg iv) on reflex-mediated IAS relaxation in each of three different intact guinea pigs.

Rectal motility was recorded with a warm water-filled balloon that was attached to flexible polyethylene tubing connected to a pressure transducer. The 1.5-cm-long balloon was introduced into the rectum 4 cm oral to the anus (Fig. 2), and the tubing was loosely fixed to a metal rod to prevent evacuation of the balloon through the anus. To record the basal rectal motility, 0.05 ml of water had been infused into the balloon. We confirmed that the balloon itself did not generate any pressure due to the elastic properties of the balloon when <2.0 ml of water was infused. Gradual and sustained rectal distension at each interval of 20 min was performed by continuously infusing 0.6 ml of warm water into the balloon at the rate of 1.5 ml/min for 24 s and by clamping the infusion tube for 4 min 36 s (total 5 min; Figs. 2 and 3). This rectal distension method simulated the rectal distension by transported and reserved feces. The rectal distension did not affect the systemic blood pressure, indicating that the stimulus was nonnociceptive. During infusion of water into the balloon up to 0.6 ml for 24 s, no reflex response was evoked. Subsequent sustained rectal distension evoked reflex responses superimposed on a sustained, passively generated pressure of 35–75 mmHg. This volume is the same as the previous one (22) and corresponds to two pieces of fresh feces.

The motility of the IAS was recorded with a custom-made strain gauge force transducer (Fig. 2) composed of a pair of needles and a base. The needles were horizontally fixed and inserted into the anus 0.5 cm oral to the anal margin. The needles were fixed on the base where two strain gauges (120 ± 0.5 Ω each) forming a half-bridge were mounted (see Fig. 2, inset). The needles moved from the left to the right according to the IAS motility, and this moved the strain gauges. At calibration, this transducer was vertically fixed at the base so that the needle on either side was upward, and 1-g weights were suspended on it. The transducer was similar to that used by Mizutani and Nakayama (11) to measure the motility of the canine IAS but was modified for use in guinea pigs. Force generated by strain in this transducer was linear, between 0 and 1.0 mm. The mean frequencies of the spontaneous motility of the rectum and the IAS without any interventions were 6.14 ± 2.54 and 10.14 ± 3.13 cycles/min, respectively, and there were significant differences (P < 0.001) between them in seven guinea pigs. The rhythm of the

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**Fig. 1. Protocols 1 and 2 for extrinsic reflex and protocol 3 for intrinsic reflex.** LCNs, section of the lumbar colonic nerves; HGNs, section of the hypogastric nerves; TH 13, transection of the thoracic cord at 13th level; PITH (S1–3), pithing sacral cords between 1st and 3rd levels; LS, lumbar sympathectomy; GUA, guanethidine (3 mg/kg iv); L-NAME, Nω-nitro-l-arginine methyl ester hydrochloride (10 mg/kg iv); L-NNA: Nω-nitro-l-arginine (10 mg/kg iv); TTX, tetrodotoxin (2 μg/kg iv); L-ARG, l-arginine (50 mg/kg iv); ATR, atropine sulfate (0.5 mg/kg iv).

**Fig. 2. Experimental settings.** R1 and R2, strain gauges. See text for details.
present IAS spontaneous motility overlapped with that in opossum IAS (20). After L-NAME, IAS relaxations were converted into contractions, whereas the rectal contractions remained, as shown in Fig. 8, A, c and B, c. We therefore judged that the motility of the IAS detected with this transducer was independent of rectal motility.

The trial for control rectoanal reflex response was repeated three times in each experiment. A constant reflex response was repeatedly produced in normal animals throughout the experiments by the present protocol without any interventions. After the control reflex response became constant, each guinea pig underwent various surgical operations. After each surgical operation or drug application, two or three trials of rectal distension for 5 min at 20-min intervals were performed and the best reflex response in two or three trials was evaluated. Mean systemic arterial blood pressure (SAP) was maintained between 100 and 150 mmHg (physiological range) throughout the experiment, and PO2, P CO2, and pH were maintained within the physiological range by changing the tidal volume and rate of artificial ventilation. The body temperature was also maintained within the physiological range at 36–37°C with a heating pad.

Figure 3 shows how we obtained the net area of each rectoanal reflex-mediated contraction of the rectum and relaxation of the IAS. The baseline was drawn on the basal pressure (A) or force level (B). AUCs, AOC, and nonreflex area were calculated by a computer-operated scanner-digitizer.

The representative sets of tracings of the rectoanal reflex (Fig. 4A, B) were elicited by the nitrergic nerve pathway. The rectal contractions were abolished by atropine or tetrodotoxin in all three intact guinea pigs tested. In three other animals, L-NAME converted the IAS relaxations into contractions, whereas the rectal contractions were unaffected (data not shown). These results indicate that the rectal contractions were elicited by the cholinergic nerve pathway and that the IAS relaxations were elicited by the nitricergic nerve pathway.

Effects of LCNs and PITH (S1–3) on extrinsic rectoanal reflex. The representative sets of tracings of the reflex-mediated rectal contractions and the IAS relaxations in control (Fig. 4A), after LCNs (Fig. 4B), and after PITH (S1–3) (Fig. 4C) (protocol 1) are shown. The
initial transient increase in rectal intraluminal pressure induced by gradual distension did not elicit the reflex response and was tetrodotoxin insensitive. This phase is excluded from the reflex area (see Fig. 3). The subsequent, sustained rectal distension evoked reflex responses superimposed on a sustained, passively generated pressure of 55–75 mmHg. The rectoanal reflex caused six phasic contractions in the rectum and simultaneous phasic relaxations in the IAS. LCNs noticeably increased amplitude in rectal contractions (reflex index from 1.0 to 1.78) and IAS relaxations (reflex index from 1.0 to 1.36) (Fig. 4B). Subsequent HGNs did not result in a further change in the reflex responses (not shown), but PITH (S1–3) markedly decreased both the rectal contractions and IAS relaxations without any effect on basal motility (Fig. 4C). The basal motility was greatly attenuated by papaverine (5 mg/kg iv).

Effects of LCNs, TH 13, and PITH (S1–3) on extrinsic rectoanal reflex. The summarized data on the effects of successive surgical operations on reflex-mediated rectal contractions and IAS relaxations evaluated by the reflex index in the same guinea pigs (n = 6; protocol 1) are shown in Fig. 5. The reflex index in the control was expressed as 1.0. LCNs significantly increased both rectal (P < 0.005) and IAS reflex indexes (P < 0.001) by approximately twofold to 2.18 ± 0.96 and 2.34 ± 0.46, but subsequent HGNs did not lead to further alteration of these indexes. TH 13 caused slight reductions in rectal and IAS reflex indexes to 1.57 ± 0.48 and 1.96 ± 0.20, but these changes were not significant. PITH (S1–3) significantly decreased rectal (P < 0.005) and IAS reflex indexes (P < 0.001) to 0.34 ± 0.24 and 0.51 ± 0.08, respectively (Fig. 5A).

When the order of HGNs and LCNs was reversed, HGNs did not affect either rectal or IAS reflex indexes but subsequent LCNs significantly increased both rectal and IAS reflex indexes by approximately twofold, to 1.90 ± 0.39 and 2.13 ± 0.44, (P < 0.001 vs. each control; n = 6; Fig. 5B). TH 13 slightly reduced the rectal and IAS reflex indexes to 1.55 ± 0.70 and 1.72 ± 0.27, but these reductions were not significant. Final PITH (S1–3) significantly (P < 0.001) decreased rectal and IAS reflex indexes to 0.36 ± 0.14 and 0.50 ± 0.10. We confirmed that initial PITH (S1–3) alone left the intrinsic rectal and IAS reflex indexes at 0.37 ± 0.17 and 0.59 ± 0.21 in a different group of six guinea pigs. There were no significant differences between initial PITH (S1–3) and the final PITH (S1–3) after the successive surgical procedure (see data in Figs. 5 and 7), indicating that the surgical procedure per se did not alter the effect of the final PITH (S1–3).

Effects of TH 13, LCNs, and PITH (S1–3) on extrinsic rectoanal reflex. Each representative set of tracings of reflex-mediated rectal contractions and IAS relaxations to near control levels (Fig. 6D). The final PITH

**Fig. 4.** A representative set of tracings of the rectoanal reflex causing contraction in the rectum (R) and relaxation in the recto-IAS in control (A), after LCNs (B), and after PITH (S1–3) (C) in protocol 1. n. Nerve. A: extrinsic reflex composed of intense pelvic-pelvic nerve reflex, weak colonic-colonic nerve reflex, pelvic nerve-supraspinal center-colonic nerve reflex, and intrinsic reflex. B: extrinsic reflex composed of pelvic-pelvic nerve reflex and intrinsic reflex. C: intrinsic reflex.
(S1–3) decreased both rectal contractions and IAS relaxations (data not shown).

Figure 7A shows the summarized data for the same guinea pigs (n = 6). TH 13 reduced (P < 0.001 vs. each control) the rectal and IAS reflex indexes to 0. HGNs did not affect the reflex index further, but subsequent LCNs significantly (P < 0.001 vs. each control) increased the rectal and IAS reflex indexes to above the control level. The final PITH (S1–3) significantly (P < 0.001) decreased both reflex indexes to 0.43 ± 0.28 and 0.37 ± 0.19. In Fig. 7B, the order of HGNs and LCNs (n = 6) is reversed from that in Fig. 7A. The result was substantially the same as that in Fig. 7A.

Effects of L-NAME or L-NNA and atropine, tetrodotoxin, or L-ARG on intrinsic rectoanal reflex. PITH and guanethidine treatment abolished typical reflex-mediated rectal contractions and IAS relaxations without any effect on basal motility (protocol 3; n = 4) (Fig. 8A, B). Only the intrinsic (enteric) inhibitory nerve pathways would be expected to remain under these conditions. L-NAME administration led to intrinsic rectal and IAS contractions (Fig. 8A, C), and these contrac-

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**Fig. 5.** A: summarized data on effects of successive surgical operations in protocol 1 on reflex-mediated contraction in the rectum and relaxation in the IAS evaluated by the reflex index in the same guinea pigs (n = 6). *P < 0.005 vs. control. **P < 0.001 vs. control. #P < 0.005. ##P < 0.001. B: only the order of HGNs and LCNs is reversed from that in A (n = 6). *P < 0.001 vs. control. #P < 0.001.

**Fig. 6.** Representative set of tracings of reflex-mediated contraction in the rectum and relaxation in the IAS in control (A), after TH 13 (B), after HGNs (C), and after LCNs (D) in protocol 2. A: extrinsic reflex composed of pelvic-pelvic nerve reflex, weak colonic-colonic nerve reflex, pelvic nerve-supraspinal center-colonic nerve reflex, and intrinsic reflex. B: extrinsic reflex composed of intense colonic-colonic nerve reflex, pelvic-pelvic nerve reflex, and intrinsic reflex. C: extrinsic reflex composed of pelvic-pelvic nerve reflex and intrinsic reflex.
tions were abolished by tetrodotoxin (Fig. 8A, d) without any effect on basal motility. These data indicate that the intrinsic excitatory and inhibitory nerves remain intact in the intrinsic rectoanal reflex pathway after PITH (S1–3) and guanethidine.

In contrast, PITH and guanethidine treatment largely decreased but did not abolish typical reflex-mediated rectal contractions and IAS relaxations without any effect on basal motility (protocol 3; n = 4) (Fig. 8B, b). Intrinsic (enteric) excitatory and inhibitory

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**Fig. 7.** A: summarized data on the effects of successive surgical operations in protocol 2 on rectal and IAS reflex indexes in the same guinea pigs (n = 6). B: only the order of HGNs and LCNs is reversed (n = 6). *P < 0.005 vs. control. **P < 0.001 vs. control. #P < 0.001.

**Fig. 8.** Two representative sets of tracings of the reflex-mediated rectal contractions and IAS relaxations and contractions in the control (a), after PITH (S1–3) + guanethidine (b), after l-NAME (c), and after TTX (d) in A and in control (a), after PITH (S1–3) + guanethidine (b), after l-NNA (c), and after atropine (d) in B in protocol 3. b: Intrinsic cholinergic excitatory reflex and nitrergic inhibitory reflex. c: Intrinsic cholinergic excitatory reflex.
nerves appear to remain in this state, because L-NNA enhanced the rectal contractions and converted IAS relaxations into contractions (Fig. 8B, c) and atropine administration abolished both rectal and IAS contractions (Fig. 8B, d) without any effect on basal motility. These results confirmed that the intrinsic excitatory and inhibitory nerves remain in the intrinsic rectoanal reflex pathway after PITH (S1–3) and guanethidine.

The summarized data for effects of guanethidine, L-NAME, and tetrodotoxin on the reflex indexes after PITH (S1–3) (reflex index = 1.0) in the same guinea pigs (n = 7) are shown in Fig. 9A. Guanethidine did not alter the indexes, but L-NAME significantly increased the rectal reflex index and changed the IAS reflex index into a negative value (reflex index = 6.13 ± 1.37 and −2.81 ± 0.92; P < 0.001 vs. L-NAME). Tetrodotoxin reduced both reflex indexes to 0 [P < 0.001 vs. PITH (S1–3) and L-NAME]. Instead of tetrodotoxin, L-ARG significantly (P < 0.01 vs. L-NAME) decreased both reflex indexes (reflex index = 0.31 ± 0.07 and −0.09 ± 0.07) after the same surgical procedure and drug treatment (protocol 3) in another series of experiments (n = 3; data not shown).

Guanethidine did not alter the indexes, but L-NNA caused a sevenfold increase in the rectal reflex index [P < 0.001 vs. PITH (S1–3) and guanethidine; reflex index = 7.25 ± 1.48] and converted the IAS reflex index into a negative value (reflex index = −2.16 ± 0.60). Atropine reduced both reflex indexes to 0 (P < 0.001 vs. L-NNA). The summarized data for effects of guanethidine, L-NNA, and atropine on the reflex indexes after PITH (S1–3) (reflex index = 1.0) in the same guinea pigs (n = 4) are shown in Fig. 9B.

### DISCUSSION

The most important finding in the present study was that the lumbar inhibitory outflow through the LCNs markedly suppressed rectoanal reflexes, causing both contractions (cholinergic) of the rectum and relaxations (nitrergic) of the IAS. Consistent with this, rectal balloon distension-mediated IAS relaxation is significantly suppressed by the α2-adrenergic agonist clonidine (28) and by lumbar sympathetic nerve stimulation (14) in the opossum. Although HGN stimulation suppressed the rectal balloon distension-induced fall in IAS pressure, rectal balloon distension caused a fall in IAS pressure without any significant change in HGN efferent activity (20). It was suggested that HGNs may not play a significant role in rectoanal reflex-induced IAS relaxation (20). Together, these data suggest the contribution of lumbar sympathetic nerves excluding HGNs, i.e., LCNs, to the rectoanal reflex in opossum. Moreover, we suggest that the inhibitory outflow from the lumbar spinal cord by way of the LCNs plays an important role in integrative control of the rectoanal reflex in the guinea pig.

**The recto-IAS reflex by rectal distension.** Rectal distension is a more physiological stimulus than electrical field stimulation, which is commonly used in in vitro studies (4, 5). Distension stimulates afferent stretch receptors in the rectum, whereas electrical field stimulation directly activates enteric nerve pathways.
Gradual, sustained rectal distension, as used in the current study, simulating the rectal distension by transported and then reserved two pieces of feces was a more physiological stimulus than those in our previous studies (22–24), in which prompt, sustained rectal distension was used. In the present study, we were able to induce constant rectal contractions and simultaneous IAS relaxations by means of the rectal distension, although the possibility that the rectal contraction evoked by rectal distension secondarily causes IAS relaxation cannot be excluded. Furthermore, spontaneous slowly migrating motor activity involving both excitatory and inhibitory enteric neurons has recently been observed in guinea pigs (3). We have tested this possibility by simultaneous recording of the motility in the colon (7–9 cm oral to the anus) and rectum (4 cm oral to the anus) (our unpublished observations). Several phasic rectal contractions were elicited by the balloon distension in the rectum, whereas the colonic motility was unchanged. It seems likely that spontaneous slowly migrating motor activity never occurred during the rectal distension without any intervention. Although further studies are needed to determine whether this motor activity could be involved in reflex-mediated contractions and relaxations, it is conceivable that major parts of rectal contractions and IAS relaxations elicited by rectal distension are nerve-mediated reflex responses.

**Rationality of the reflex index.** In the present study, we adopted a reflex index to quantitatively evaluate reflex-mediated rectal contractions and IAS relaxations and contractions. There are many studies, including our own (21), that evaluate gut motility quantitatively, but the reflex responses are composed of various wave patterns, so that the simple evaluation of the amplitude and frequency of each wave is not appropriate in evaluating the rectoanal reflex. The present reflex index is a relevant assessment because it corresponds to the power evacuating fecal contents, and it would be appropriate for quantitative evaluation of the reflex response composed of various wave patterns.

**Extrinsic lumbar inhibitory reflexes through LCNs.** The rectoanal reflex caused by both rectal contractions and IAS relaxations were doubly enhanced after LCNs (not HGNs), indicating that the lumbar outflow through the LCNs halved the rectoanal reflex in the guinea pig. This finding is novel and somewhat different from that in our previous reports (22–24). It seems likely that the inhibition from the pons to lumbar nerve efferent activity was less potent in the present study than in our previous studies (22–24). The difference between our previous result and the present one may involve the different methods of rectal distension that were employed (prompt distension vs. gradual, sustained distension) as discussed below.

**Extrinsic sacral excitatory reflexes.** Final PITH (S1–3) in protocols 1 and 2 largely attenuated the rectal contraction and IAS relaxation, indicating that the sacral excitatory reflex through the pelvic nerves causes the rectal contraction and IAS relaxation. This is partly supported by previous findings indicating that synaptic inputs to myenteric neurons in the guinea pig rectum from pelvic nerves have been identified (27). The enhanced rectal contraction and IAS relaxation after LCNs (see Fig. 5) were only slightly decreased after TH 13, indicating that the supraspinal facilitation on the pelvic nerve outflow does not play an important role in the present reflex mechanism. This is consistent with our previous results on the rectoanal reflex in the guinea pig (22).

**Supraspinal inhibition on lumbar inhibitory outflow.** The reflex-mediated rectal contraction and IAS relaxation appeared to be abolished without any fall in SAP after TH 13. As mentioned above, the supraspinal facilitation on the pelvic nerve outflow does not greatly contribute to the present reflex mechanism. The restoration of both rectal contractions and IAS relaxations after the following LCNs indicates that the descending nerve pathway, possibly from the pons (23), contributes to suppress the lumbar inhibitory outflow in the rectoanal reflex. This descending nerve pathway from the pons is activated by pelvic afferent nerve activation (23). The pelvic afferent nerve is activated by rectal distension (22). The intensity of the descending inhibition seems to depend on the method of rectal distension. It seems likely that the present gradual and sustained rectal distension activates pelvic afferents less potently than the prompt rectal distension does (22).

**Role of the enteric nitrergic nerve pathway in the intrinsic reflexes.** A recent study suggested the possibility that nitrergic neurotransmission is involved in electrical field stimulation-induced nonadrenergic, noncholinergic relaxation of the rat rectal circular muscle (26) and IAS (4). In the human distal rectum, nitrergic axons enter shunt fascicles that descend into the anal canal, where they ramify into and throughout the IAS (15). It is reasonable, therefore, that the nitrergic nerve pathways in the rectum and IAS are activated by the rectal distension. The extrinsic sacral excitatory and lumbar inhibitory nerve pathways (adrenergic nerve fibers) in the rectum and IAS were impaired after PITH (S1–3) and guanethidine (9), and only intrinsic (enteric) excitatory and inhibitory nerve pathways remained. Consequently, both rectal and IAS reflex indexes were greatly decreased (see Figs. 5 and 7) but were not 0. L-NAME or L-NNa enhanced the intrinsic rectal contractions and converted IAS relaxations into contractions or evoked IAS contractions. These intrinsic reflex-mediated rectal and IAS contractions were abolished by tetrodotoxin or atropine or were greatly decreased by L-ARG. Moreover, these findings indicate that enteric cholinergic excitatory and nitrergic inhibitory nerve pathways contribute to the intrinsic rectoanal reflex, although it is unknown whether intrinsic reflex-mediated IAS relaxations are evoked by rectal distension or are secondarily evoked by rectal distension-induced rectal contraction.

L-NNa has been reported to convert the relaxations in the isolated human rectum into cholinergic contractions and to convert the relaxations in the isolated human IAS into α-adrenergic contractions (5). In the...
REFERENCES