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Sympathetic and Parasympathetic Nervous Activity in the Proximal-distal Colonic Reflex of Dogs

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ABSTRACT. Relationships were examined between outflows of the lumbar colonic nerve (LCN) and rectal branch of the pelvic nerve (RB) and distal colonic motility (DCM) during proximal colonic distension in anesthetized and decerebrate dogs. Distension of the proximal colon (PC) reflexively elicited an increase in outflows of both the LCN and RB, often accompanied with an initial inhibition followed by an augmentation of DCM. Increasing responses in both the RB outflow and DCM were demonstrable after transection of the brain stem at the subcollicular level, but they disappeared after midpontine transection just caudal to the pontine defecation reflex center. The afferent pathways mainly involved the vagus nerves and partly the abdominal sympathetic nerves. While the increase in the outflow of the LCN and inhibition of DCM remained after bilateral cervical vagotomy as well as spinal transection (Th 6-7), but they disappeared after destruction of the spinal cord. These results indicate that the pontine defecation reflex center is an indispensable to the proximal-distal colonic augmentative reflex, while the inhibitory reflex originates in the spinal cord.

Key words : Proximal-distal colonic reflex — Pelvic nerve — Lumbar colonic nerve — Vagus nerve — Pontine defecation reflex center

Semba (1955)[1] reported that inhibition of colonic motility was induced in dogs by activation of the reflex from the proximal colon (PC) to the distal colon (DC) (proximal-distal colonic reflex), and that afferent and efferent pathways of the reflex involved the lumbar colonic nerve (LCN). Then Ohashi (1969)[2] found in dogs that colonic motility was augmented by distension of the PC via the reflex center in the spinal cord. However, Kreulen and Szurszewski (1979)[3] reported that a colo-colonic inhibitory reflex was mediated through pathways in the prevertebral ganglia in guinea-pigs in the *in vitro* experiments.

Outflow of the pelvic nerve and distal colonic motility have been shown to increase by distension of the esophagus,[4] stomach[5] and small intestine.[6] These augmentative effects are induced via the pontine defecation reflex center.[7,8] The purpose of the present experiment was to examine the relationship between outflows of the LCN and rectal branch of the pelvic nerve (RB) and distal colonic motility during the proximal-distal colonic reflex in dogs, to define the reflex pathways and to determine how the pontine defecation reflex center relates to this reflex.

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METHODS

Twenty-three dogs of both sexes (4–12 kg) were used in this experiment. A balloon, 3 cm long and 5 ml in volume, was inserted into the distal colon (DC) with the tip of the balloon 10 cm from the anus. Another balloon, 4 cm and 5 ml, was inserted into the proximal colon (PC) with the tip of the balloon located about 8 cm caudal to the incision in the ceacum. Motility of the DC and PC was recorded by the balloon–pressure transducer method. The same balloon used to record motility of the PC was used for distension. The DC was severed from the PC in all but three dogs (two in the first group and one in the second group) 4 cm oral to the point where the inferior mesenteric artery enters the DC.

The first group of 18 dogs were decerebrated. The rectal branch (RB) of the unilateral pelvic nerve was isolated and prepared for recording by a midline incision in 13 dogs of this group. Bipolar electrodes of platinum wire were used for recording the nervous outflow. The outflow was amplified with an RC-coupled amplifier and converted into a frequency histogram of 200 msec or 500 msec bins with a spike counter. Both hypogastric nerves (HGN) and the lumbar colonic nerve (LCN) were severed in 12 dogs, and only the HGN were severed in one. As for the remaining five dogs in this group, in which only colonic motility was recorded, only the LCN was cut in two dogs, and both the HGN and LCN were left intact in the other three.

In the other group of 5 dogs, which were anesthetized with morphine hydrochloride (3 mg/kg, i.v.) and urethane (0.25 g/kg, i.m.), the LCN was cut at the point where it enters the distal colon, and outflow of its branch was recorded by the same method as for the RB. The pelvic nerve and HGN were intact in this group, except that the unilateral hypogastric nerve was cut in one dog.

Cervical vagi were cut in 12 dogs, 8 in the first group and four in the second group. The vagus nerve was sometimes prepared for electrical centripetal stimulation with a bipolar electrode of silver wire (10–20 V, 1 ms, 10–20 Hz).

The dorsal surface of the brain stem was exposed by a craniectomy, and cut at the levels of the subcolliculus and midpons (15 mm oral to the obex) in 10 dogs of the first group.

In the second group, both the 6–7th thoracic cord and the 6–7th lumbar cord were exposed by lamiectomy. The spinal cord was cut first between the 6th and 7th thoracic vertebra, and then the lower spinal cord caudal to the 6th thoracic vertebra was destroyed with a steel rod 5 mm in diameter in 3 dogs, while in 2 dogs the spinal cord was locally anesthetized by subdural infusion of 3–4 ml of 1% xylocaine solution from the 6th thoracic level to the lower lumbar region.

The anal mucosa was often stimulated mechanically with an acrylic resin rod 1 cm in diameter. The animals were paralyzed with gallamine triethiodide (1 mg/kg) and ventilated artificially through a tracheal cannula at a rate of 20 strokes/min and a tidal volume of 150–200 ml. The blood pressure was monitored at the right femoral artery, and the body temperature was maintained at about 36°C throughout the experiment. Morphine hydrochloride (1 mg/kg, i.v.) was occasionally added.

RESULTS

The outflow of the pelvic nerve during distension of the PC and effect of brain stem transection on the proximal-distal colonic reflex

Efferent discharges of the RB of the pelvic nerve were increased by distending the PC in all 12 dogs studied. When the intraluminal pressure of the PC was more than 10 mmHg, the outflow of the RB began to increase in most cases (Fig. 1A), while the distal colonic motility was affected by the stimulation of more than 30 mmHg in intensity (Fig. 1C-E). The threshold for the increase

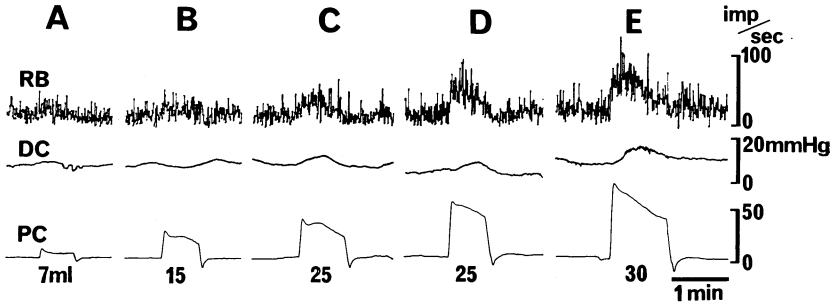

Fig. 1. Effect of PC distension on RB outflow in the pelvic nerve. Decerebrate dog. The HGN and LCN were severed, and the colon was cut between PC and DC. The curves from top to bottom : the efferent activity of the RB of the left pelvic nerve (bin width, 500 msec) ; intraluminal pressure of the DC and intraluminal pressure of the PC. The distension volume is shown under each PC curve. The same abbreviations are used in the following figures.

of the nervous discharge was about 5 mmHg (5-6 ml). Furthermore, as shown in Figure 1, the higher the stimulus intensity became, the more the discharge increased. Such remarkable increases in the outflow of the RB, which were induced by proximal colonic distension of more than 50 mmHg, sometimes resulted in defecation with a large contraction of the DC (A of Figs. 2-4).

The brain stem was transected in 5 dogs of this group. When the brain stem was cut at the subcollicular level, the increasing responses of the nerve and colonic motility during the stimulation remained (Fig. 2B), but after a midpontine transection, no increasing response in the nervous outflow or motility was elicited even when the pressure of the stimulus was more than 50 mmHg (Fig. 2C). Mechanical stimulation of the anal mucosa still induced increases in both responses. Additionally, spontaneous activity of the nerve generally decreased after the transection of the midpons.

The effect of vagotomy and centripetal stimulation of the cervical vagus nerve on RB outflow and DC motility

Bilateral cervical vagotomy completely abolished the RB response to distension of the PC (Fig. 3B) in 7 out of 8 dogs. The augmentative reflex effect was greatly reduced, but did not disappear completely, in the nervous response in one dog (Fig. 4B). The reduced response in this dog was still induced after subcollicular transection (C), but it was completely abolished

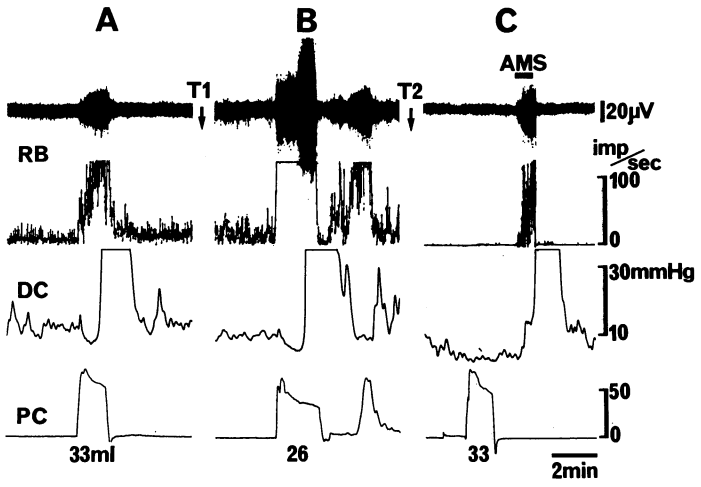

Fig. 2. Effect of brain stem transection on the proximal-distal colonic reflex. Decerebrate dog. The HGN and LCN were cut, and the PC and DC were separated. The upper curves show the outflow of the RB (bin width in the second one, 200 msec). A, control ; B, 1 hr after subcollicular transection (T1) ; C, 35 min after midpontine transection (T2). The RB responded well to mechanical stimulation of the anal mucosa (AMS) in C, indicating that the spinal level of the animal was intact. The same abbreviations are used in the following figures.

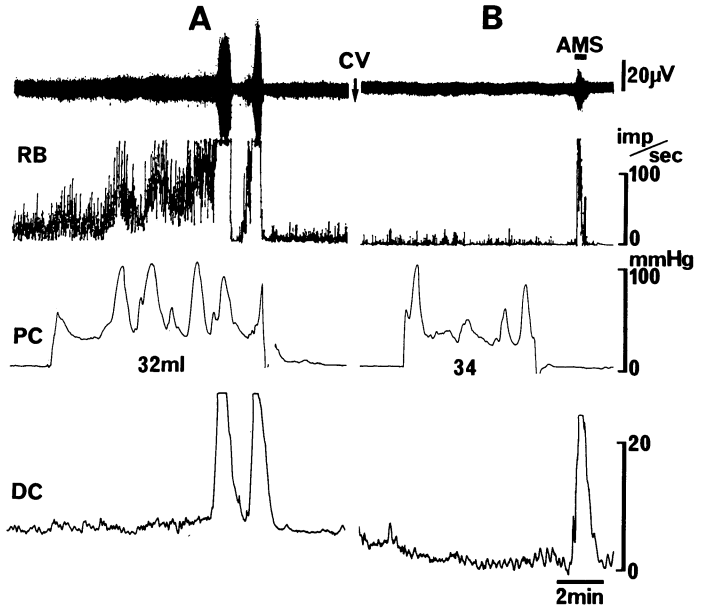

Fig. 3. Effects of cervical vagotomy on the proximal-distal colonic reflex. Decerebrate dog. The HGN and LCN were cut, and the colon was separated. The upper two records show the outflow of the RB (bin width of the second one, 200 msec). Bilateral cervical vagotomy was done between A and B indicated by CV with an arrow. AMS was performed in B.

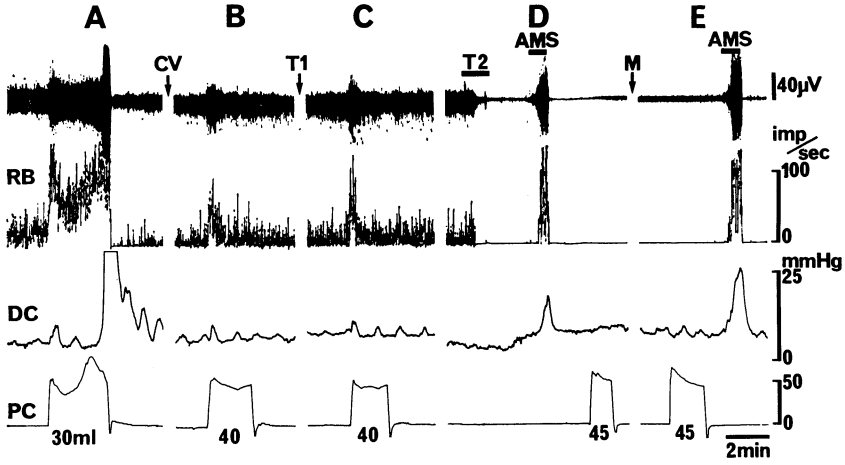

Fig. 4. Effect of brain stem transection on reflex response of RB outflow after cervical vagotomy. Decerebrate dog. Both the HGN and LCN were cut. The colon was divided. A, control. Reflex effects by distension of the PC : B, after vagotomy (CV) ; C, after subcollicular transection (T1) ; D, after midpontine transection (T2) ; E, 1 hr after T2. Morphine hydrochloride (0.5 mg/kg) was applied between D and E, but AMS was effective. No reflex effect was produced by distension of PC.

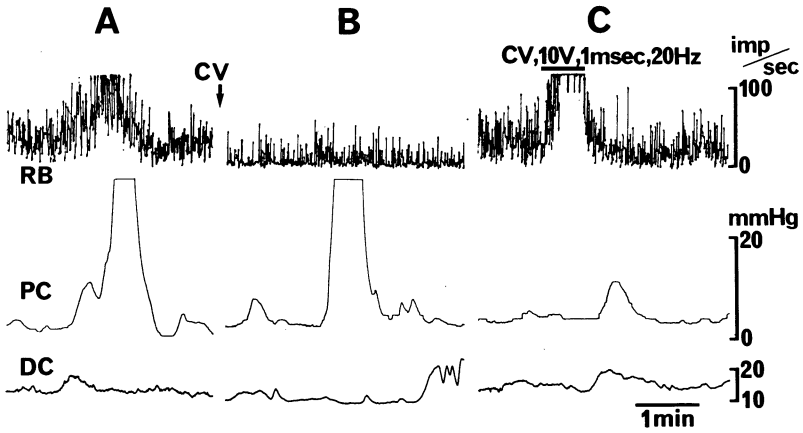

Fig. 5. Effect of marked contraction of the PC and centripetal stimulation of the vagus nerve on RB outflow. Decerebrate dog. The HGN and LCN were cut. The colon was not divided in this dog. Marked spontaneous contraction of the PC was seen in A and B. The increase in the outflow of the RB was synchronized with spontaneous contractions of PC in A, but not in B after cervical vagotomy (CV). The left cervical vagus nerve was stimulated centripetally (10V, 1 msec, 20 Hz) during the period indicated by a bar in C. Note that the right vagus nerve was still intact in C.

after midpontine transection (D,E). The increased outflow in the RB was also elicited by spontaneous contractions of the PC (Fig. 5A), but such increased outflow was abolished by bilateral severance of the cervical vagus nerves (B).

When the severed cervical vagus trunk was stimulated centripetally, the RB outflow increased remarkably, and the distal colonic motility was augmented (Fig. 5C) just as when the PC was distended.

The sympathetic outflow during distension of the PC and the effect of anesthesia or destruction of the spinal cord

The outflow of 13 branches (out of 31 in 5 dogs of the second group) of the LCN increased during mechanical stimulation of the anal mucosa, so the responses of these branches to proximal colonic distension were tested. The outflow of all 13 branches of the LCN increased by the distension (Fig. 6A). This increased response of the nerve did not change after cutting the cervical vagi, and even after cutting the 6th thoracic spinal cord in four dogs tested (B,C). However, when the spinal cord was anesthetized by subdural infusion of xylocaine from the 6th thoracic area to the caudal lumbar region, the reflex response completely disappeared, not only in the nervous outflow, but also in the motility of the DC (D). The reflex effects in both the outflow of the LCN and distal colonic motility recovered partially after the spinal washing with Tyrode's solution (E). The same area of the spinal cord as that anesthetized was destroyed in three dogs of this group. The reflex responses disappeared completely in these dogs as well.

Reflex effects on distal colonic motility during distension of the PC

Decerebrate dogs : When the PC was distended 20–30 ml (over 40 mmHg in intraluminal pressure), motility of the DC was enhanced with or without an initial inhibition in sixteen out of 18 decerebrate dogs (Figs. 1C–E and 2A). In some cases, such enhanced motility developed to a propulsive defecation wave following vigorous outflows of the RB in pelvic nerves (A of Figs. 2–4). The augmentative effect on the DC by distension of the PC was abolished after cervical vagotomy, though the outflow of the pelvic RB could be increased in one dog (Fig. 4B,C).

In most of these dogs, the PC and DC were separated to exclude descending inhibition via the intrinsic reflex in the colonic wall.[9,10] An inhibition of motility of the DC following distension of the PC was usually abolished by cutting the HGN and LCN as well as by local spinal anesthesia, but not by cervical vagotomy (Fig. 6D). In rare cases, an initial inhibition followed by a propulsive wave was elicited despite the severance of the HGN and LCN and colonic division (Fig. 2A). No clear effect was observed on the motility in the two colonic sympathectomized dogs whose colon was severed.

Anesthetized dogs : In dogs anesthetized with morphine and urethane, the augmentative effect on the motility of the DC by distension of the PC was weaker than in decerebrate dogs, but the inhibitory effect on the DC was easily elicited by distension of the PC. However, distal colonic motility in two out of 5 dogs was constantly inhibited by distension of the PC. In the other three dogs of the group, the reflex effect on colonic motility was variable : inhibitory,

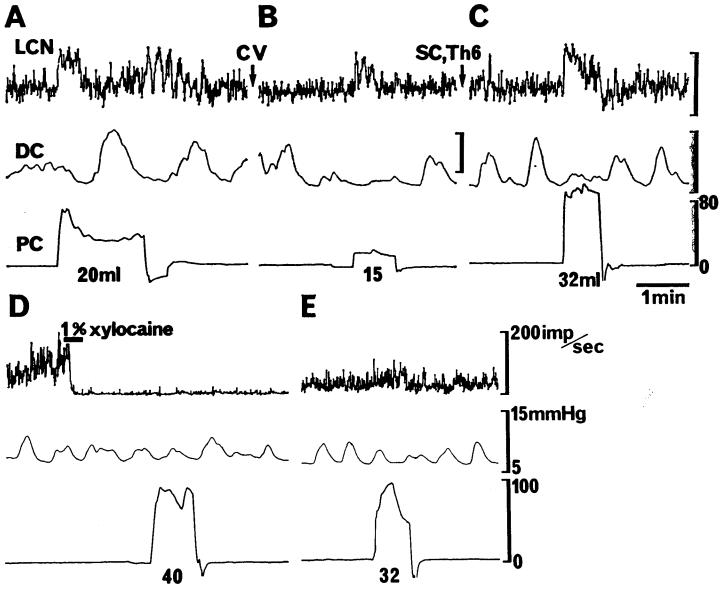

Fig. 6. Effect of vagotomy and spinal anesthesia on LCN outflow by distension of the PC. The dog was anesthetized with morphine and urethane. The HGN and pelvic nerves were intact. The PC was separated from the DC. The top traces show efferent activity of the LCN. A ; control. B ; reflex effects by distension of PC after cervical vagotomy (CV). The spinal cord was cut at the 6th thoracic cord (SC, Th 6) between B and C. C ; reflex effects 2 hr after the spinal cut. During the period of the bar in D, the spinal cord caudal to the 6th thoracic area was locally anesthetized with 1 % xylocaine. E ; partial recovery of reflex effects 90 min after washing the spinal cord with Tyrode's solution.

augmentative or both (the colon of one dog was not divided).

DISCUSSION

Augmentation of the outflow of the RB of the pelvic nerve was induced through an extrinsic reflex by distension of the PC to an intraluminal pressure of over 5 mmHg (5-6 ml in volume). The response of the nerve sometimes grew strong enough to produce a large contraction of the DC (A of Figs. 2-4). The response completely disappeared or was remarkably depressed after cutting the cervical vagus nerve. Therefore, it is reasonable to conclude that the afferent pathways of the proximal-distal colonic augmentative reflex mainly involved the vagus nerves, and probably to some extent the abdominal sympathetic nerves. Semba *et al.* (1955)[11] reported that centripetal stimulation of the splanchnic or mesenteric nerve augmented distal colonic motility in anesthetized or decerebrate dogs.

Furthermore, the present study demonstrated that the augmentative response of the RB of the pelvic nerve to distension of the separated PC was completely disappeared after the transection at the midpontine level, but not after subcollicular transection. Thus, the vagal afferent impulses from the PC may

activate the pontine defecation reflex center. The command of the reflex center descends along the reticulo-spinal pathway in the medulla oblongata and the spinal cord, and it fires the sacral parasympathetic neurons innervating the colonic muscles. Similar augmentative responses of the RB have been reported in decerebrate dogs during activation of esophageal-colonic,[4] gastro-colic[5] and intestino-colonic[6] reflexes. Such responses were shown to disappear after midpontine transection as well. Ohashi[2] described the existence of an augmentative reflex in chloralose anesthetized dogs besides an inhibitory one in motility of the DC by proximal colonic distension. He concluded that the augmentative response was caused by the pelvico-pelvic reflex. The present results, however, show that the response was due mainly to the vago-pelvic reflex, and may be partly to the sympathico-pelvic reflex.

The augmentative response in distal colonic motility was induced more vigorously and frequently in decerebrate dogs than that in anesthetized dogs, in which an inhibitory response was frequently elicited. This result suggests that anesthesia affects the excitability of neural elements in the medulla oblongata and the pons, which involve excitatory pathways, much more than that in the spinal cord, which involves inhibitory pathways. Although previous authors[12,13] have reported that no excitatory effect was elicited on motility of the DC or on outflow of the RB of the pelvic nerve by centripetal stimulation of the cervical vagus nerve, this lack of response might be caused by anesthesia.

An inhibitory response in distal colonic motility to proximal colonic distension has also been reported by previous authors,[1-3] and enhanced firing of the LCN to distension of the PC was observed in spinal cats.[13] The present experiments on anesthetized dogs confirmed a similar reflex effect in that the outflow of the LCN was increased by distension of the PC. The inhibitory response is known to be due to a sympatho-sympathetic response via the spinal cord.[14,15] However, it had been generated a long controversy as to whether the reflex is exclusively spinal or if it can still occur after prevertebral ganglia decentralization. Recently, Kreulen *et al.*[3] found in the *in vitro* experiments with guinea-pigs that distension of an orad segment of the colon produced inhibitory responses in a caudad one, and concluded that the inhibitory reflex was mediated through pathways in the prevertebral ganglia.[16] In this experiment, thoracic (Th 6-7) spinal transection did not affect the reflex response in the outflow of the LCN (Fig. 6C). However, after local anesthesia or destruction (from Th6 to L7) of the spinal cord, even spontaneous firing of the LCN almost completely disappeared, and the reflex response to distension of the PC completely disappeared, in spite of a higher stimulus intensity (more than 90 mmHg). Thus it is concluded that the proximal-distal colonic inhibitory reflex is induced mainly via the spinal cord and that the spontaneous outflow of the LCN to the colon might be generated via the spinal cord.[17] The discrepancy among these results in guinea-pigs,[3] cats[17] and this experiment may be caused by the difference in species.

Vagotomy did not affect the reflex firing of the LCN during the reflex. This result suggests that the abdominal sympathetic nerve, but not in the vagus nerve, is involved in afferent pathways of the response.

It was observed in this study that the outflow of the LCN increased during the defecation reflex, though de Groat *et al.*[13] reported that the outflow did not

change in cats. The outflow also increased by mechanical stimulation of the anal mucosa via the afferent pathway of the pudendal nerve, and furthermore, when a large contraction of the PC was induced occasionally, the outflow increased simultaneously with the contraction. Thus firings of both the pelvic nerve and the sympathetic nerve increase during activation of the defecation reflex. This result suggests that a large contraction of the colon during the activation of the defecation reflex is induced because the facilitated effect of the pelvic nerve is stronger than that of the sympathetic nerve.[18)]

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