Neural Control of the Internal Anal Sphincter Motility

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Abstract

Control mechanism of smooth muscle movement of the internal anal sphincter (IAS) by enteric and extrinsic neuvous systems in the dog was investigated. Responses of IAS muscle strips to electrical field stimulation (EFS) and neurotransmitter agents were recorded *in vitro*. The contraction response to norepinephrine or to EFS was inhibited by phentolamine. The relaxation induced by EFS was not affected by phentolamine, propranolol or atropine. The mechanical activity of smooth muscle in colon and anorectum during spontaneous defecation was recorded using strain gauge force transducers. The colon and anorectum showed the characteristic motility pattern during defecation: 1) The giant migrating contraction of the colon propagated to the rectum, 2) The relaxation of the rectum prior to the contraction, and 3) The IAS muscles continued to relax while the giant contractions of the colon were migrating to the rectum. Sacral nerves were stimulated electrically and the responses of smooth muscles in the rectum and IAS were recorded. The sacral nerve stimulation induced a relaxation followed by contraction of smooth muscle in the rectum and the relaxation in IAS. The mechanical responses of smooth muscle in the IAS were modulated by α-adrenergic excitatory and non-adrenergic, non-cholinergic inhibitory nerves. During defecation, the relaxation of IAS smooth muscle was associated with a characteristic motility pattern of the colon and anorectum. The enteric nervous systems may be organizing the motility of these muscles by way of the motor neurones under the control the extrinsic nervous systems.

Key words: internal anal sphincter, neural control, defecation, non-adrenergic non-cholinergic inhibitory nerve, sacral nerve

Introduction

The internal anal sphincter (IAS) locates at the most distal part of gastrointestinal tract, and is formed by thickened circular smooth muscle coat. At rest, the IAS smooth muscles are totally contracted state, and this tonic contraction is responsible for a part of the closure of the anal canal. Although it continues to contract to maintain continence, during defecation it needs to relax to allow passage of the colonic content through the anal canal. The inflation of the balloon in the rectum induces an inhibitory response in tonically contracted IAS smooth muscle, and

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this is considered the mechanism for facilitating the defecation (Gower, 1877).

The accommodation and defecation responses in the human rectum have been observed using manometry (Duthie, 1975). With persistent inflation of an intra-rectal balloon, an increase in pressure within the rectal ampulla is maintained for a minute or more and then decreased to the preinflation levels. When the rectal volume is increased rapidly for a shot period of time, the accommodation responses fail, leading to urgent emptying of the rectum. Large amplitude contractions migrating through the colon are associated with defecation, and the contraction is called giant migration contraction (Karus and Sarna, 1987). The coordinated motility of the colon, rectum and IAS during spontaneous defecation is also demonstrated in dogs (Matsufuji *et al.*, 1988).

In addition to the enteric nervous system, the activity of IAS smooth muscle is also affected by the extrinsic nervous systems such as sympathetic and parasympathetic nerves. Ihara and Takahira (1984) demonstrated that the relaxation of IAS smooth muscle is elicited by the stimulation of sacral nerves. The functional importance of sacral nervous system in the defecation has also been recognized (DeGroat and Krier, 1978). It is assumed that the motor coordination of muscles between IAS and proximal intestine is controlled by these peripheral nervous ststems.

The present experiments were aimed to investigate how enteric and extrinsic nerves control the motility of IAS. The motor neurones affecting the IAS were identified *in vitro* and the roles of the sacral nerves on the coordinated process between the IAS and the proximal viscera in defecation were investigated *in vivo*.

Materials and Mehtods

The present study was performed under approval of Animal Experiment Committee of Keio University School of Medicine (permission number, 990164). The animals were kept in the Experimental Animal Center of Keio University, and were treated along the ethical codes of the Center.

Five mongrel dogs were sacrificed with intravenous infusion of high doses of sodium pentobarbital solution (Nembutal, Abbot Laboratories, Chicago, IL., 30 mg/Kg i.v.), and the anus was removed with surrounding tissues. The muscle strips were obtained from the IAS immediately and suspended vertically in the organ bath containing Krebs buffer solution containing NaCl 118 mmol/L, $NaHCO_3$ 25 mmol/L, KCl 4.8 mmol/L, KH_2PO_4 1.2 mmol/L, choline chloride 20 mmol/L and glucose 11 mmol/L. The solution was aerated with a 95% O_2 + 5% CO_2 gas and kept at 37°C. Resting tension was adjusted to approximately 1.0 g. Muscle contraction was recorded isometrically using force transducer (Model SB-IT-H, Nihon Kohden, Tokyo, Japan), and displayed on a polygraph recorder. The platinum electrodes were placed both sides of the muscle strip, and were stimulated by square waves delivered from an electrical stimulator (SEN-7203, Nihon Kohden, Tokyo, Japan), through isolating unit (SS-403-J, Nihon Kohden, Tokyo, Japan). The rectangular pulses of 50 V intensity, 0.5 ms duration, and 3 to 15 Hz frequency were applied to muscles transmurally. Some neurotransmitter agents were added to the organ bath solution in order to stimulate smooth muscles.

Five mongrel dogs, weighting about 10 Kg, were anesthetized with sodium pentobarbital. Four strain gauge force transducers, 13 mm × 8 mm (F-121S, Star Medical, Tokyo, Japan) were attached to the proximal colon, distal colon, rectum and IAS. Laparotomy was performed through a midline incision and three strain gauges were implanted on the serosal surface of the colon and rectum. The proximal colon gauge was sutured on to the colon 5 cm distal to the ileocecal junction. The distal colon gauge was placed 10 cm proximal to the peritoneal reflection. The rectal gauge was placed on the colon at the peritoneal reflection. Another strain gauge was sutured on to the IAS. The anal mucosa was incised transversely at the pectinate line and the submucosal space was dissected to allow implacement of the strain gauge. The strain gauge was sutured directly on to the IAS and the mucosal incision was closed with absorbable sutures. The strain gauge axis was oriented along the circular muscle axis. The lead wires from the strain gauges were brought out between the shoulders through a subcutaneous tunnel. These were connected to Wheatstone bridge boxes (FB-01, Star Medical, Tokyo, Japan), and the electrical signals were recorded using a polygraph (Nihon Kohden, Tokyo, Japan). The dog was weared with a jacket to protect the dislodging of lead wires and also against scratching. Atropine sulfate (0.01 mg/Kg) was injected just before the implantation, to minimize shortening of colon. The dogs were allowed to recover for 14 days after the surgery.

Under general anesthesia using sodium pentobarbital, the mechanical and electrical responses of rectum and IAS smooth muscles produced by electrical stimulation of sacral nerves were recorded in five mongrel dogs. Two force transducers were attached to the rectum and the IAS, as explained above. Bipolar platinum electrodes (Yufu, Tokyo, Japan) were inserted into IAS and external anal sphincter muscles to record their electric activity. Anal canal pressure was recorded using a sleeve sensor hydrostatic pressure monitoring system (KY anorectal manometry probe, Yufu, Tokyo, Japan). The electro-mechanical signals from these are recorded using a polygraph. Laminectomy was carried out by exposing sacral nerves between the 7th lumber vertebra and the 3rd sacral vertebra. A hook-type bipolar tungsten electrode was brought into contact with the sacral nerve, and the periphery of which was electrically insultated by filling with liquid paraffin. The left second sacral nerve was stimulated using the same devices as descrived above. The nerves were stimulated with rectangular pulses of 0.5 mA intensity and 0.5 ms duration, at a frequency of 10 Hz.

Chemicals used were acetylcholine chloride (Sigma, St. Louis, MO, USA), atropine sulphate (Sigma, St. Louis, MO, USA), *l*-norepinephrine hydrochloride (Sigma, St. Louis, MO, USA), phentolamine mesylate (CIBA Geigy, Switzerland) and propranolol (Sigma, St. Louis, MO, USA).

Results

Responses of IAS muscle strips to electrical field stimulation and to neurotransmitter agents in vitro

Muscle strips of IAS showed spontaneous activities with phasic contractions, forming a waxing and waning pattern, and the frequency varied between 3 and 10 cycles per min. All strips (n=5) contracted by stimulation with *l*-norepinephrine (10^{-7} M, 10^{-6} M). The responses to *l*-norepinephrine were inhibited by phentolamine (10^{-5} M). Isoproterenol (10^{-6} M) produced no

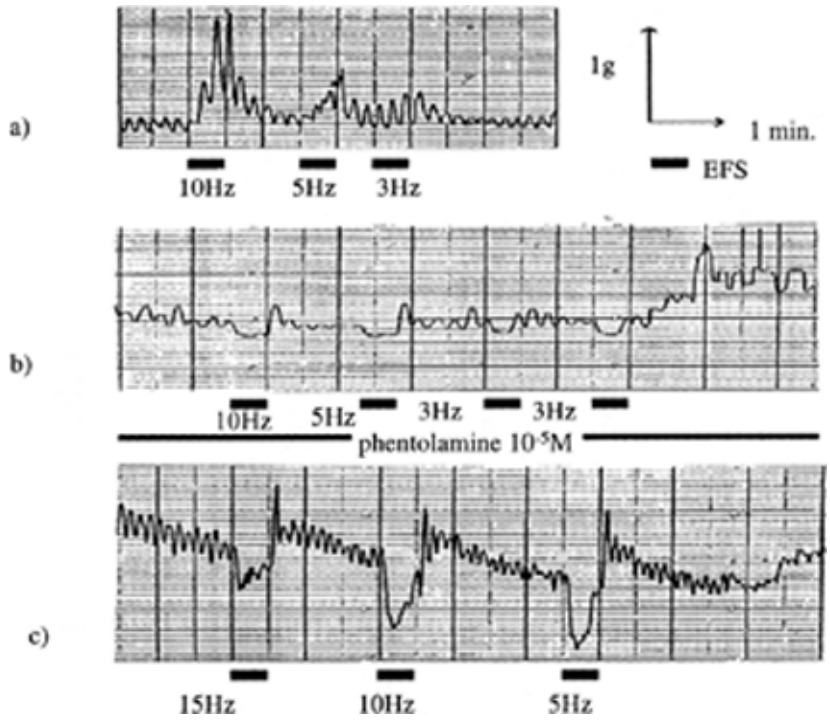

Fig. 1. Isometric mechanical responses produced by EFS in smooth muscle strips isolated from internal anal sphincter (IAS). Mechanical responses were produced by EFS in the absence (a and c) and presence of 10^{-5} M phentolamine (b). EFS: 50 V intensity, 0.5 ms duration, pulses at 3–15 Hz frequency (indicated by a bar under each record).

response in four strips and relaxation in one strip. Acetylcholine (10^{-7} M, 10^{-6} M) produced relaxation in two muscle strips and contraction in one strip. Electrical field stimulation (EFS) produced contraction response in four muscle strips and relaxation response in one strip. The contraction response was abolished by phentolamine (10^{-5} M), leaving the relaxation response unaltered (Fig. 1, a and b). In some preparations, EFS produced a sustained relaxation, and rebound contraction was followed at the cessation of EFS (Fig. 1, c). The relaxation responses induced by EFS were not affected by phentolamine (10^{-5} M), propranolol (10^{-5} M) or atropine (10^{-5} M) (Fig. 2).

Motility of colon and anorectum muscles during spontaneous defecation in conscious dog

Forty episodes of spontaneous defecation were recorded. Among them, thirty-six (90%) episodes of defecation were associated with the giant contraction (GC) of colon or rectum, which were propagated distally to the rectum or to the IAS. The rectum showed a relaxation followed by GC, which was a rectal characteristic movement. The IAS relaxed during the migration of GCs in the colon, and it remained relaxed until the GCs propagated to the rectum. The fecal material was expelled during the migration of the GC, and the evacuation was completed when the traveled GC reached the rectum (Fig. 3).

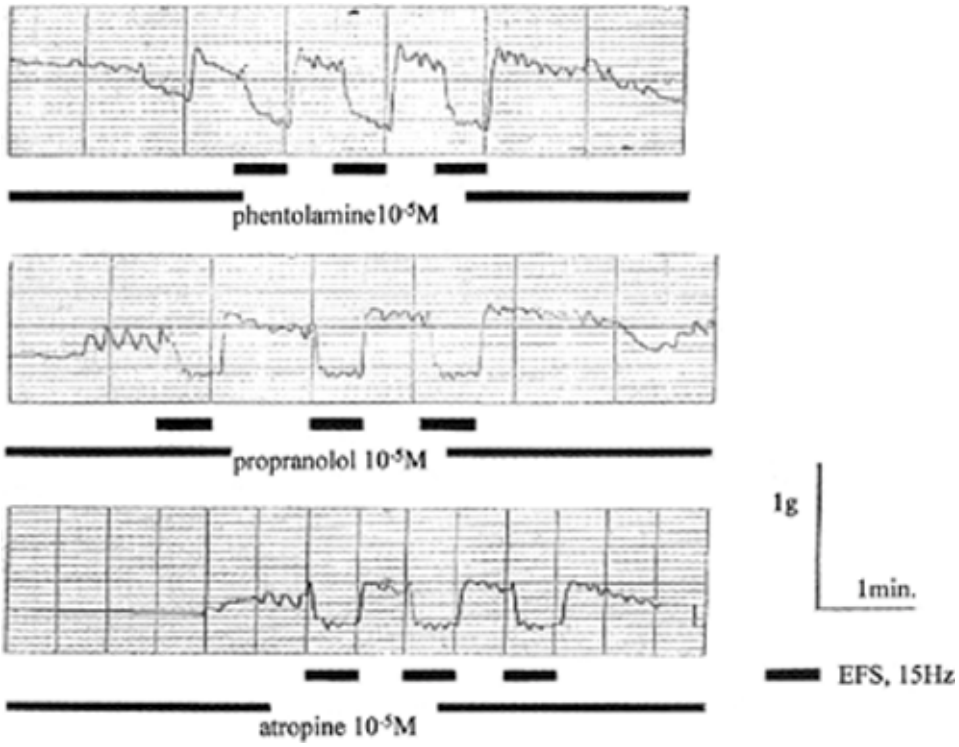

Fig. 2. Effects of phentolamine, propranolol and atropine on relaxation responses produced by nerve stimulation in smooth muscles isolated from dog internal anal sphincter (IAS). Electrical field stimulation (EFS; square pulses of 50 V intensity, 0.5 ms duration, 15 Hz frequency) was applied at the bar under each record, in the presence of 10^{-5} M phentolamine, 10^{-5} M propranolol or 10^{-5} M atropine.

The activity of colon was monitord for a long period of time while the dog was in the cage. The sum of the recording time of each dog amounted to 44 hours, during which 25 GCs occurred in the distal colon. Nine GCs appeared as single contraction. Sixteen GCs were connected to form six clusters. Eight out of 25 GCs were reached to the rectum, and induced a defecation. Seventeen GCs, which did not migrate to the rectum, did not associate with evacuation (Fig. 4).

Responses of anorectum muscles to electrical stimulation of sacral nerves in vivo

Electromyogram (EMG) recorded from smooth muscle of the IAS showed that the activity was pahsic waves, called slow waves, with the frequency ranging between 15 and 24 per min. During stimulation of the left second sacral nerve, the EMG recorded from the external anal sphincter muscle showed an elevated electrical activity, which was associated with an increased anal pressure. When the stimulation was finished, the anal canal pressure started to decrease. While the pressure was decreasing, the IAS muscle showed a relaxation with a diminished generation of slow waves. The tone of the sphincter muscle and the pressure of anal canal turned to increase to the previous level, with associated appearance of electrical activities in IAS

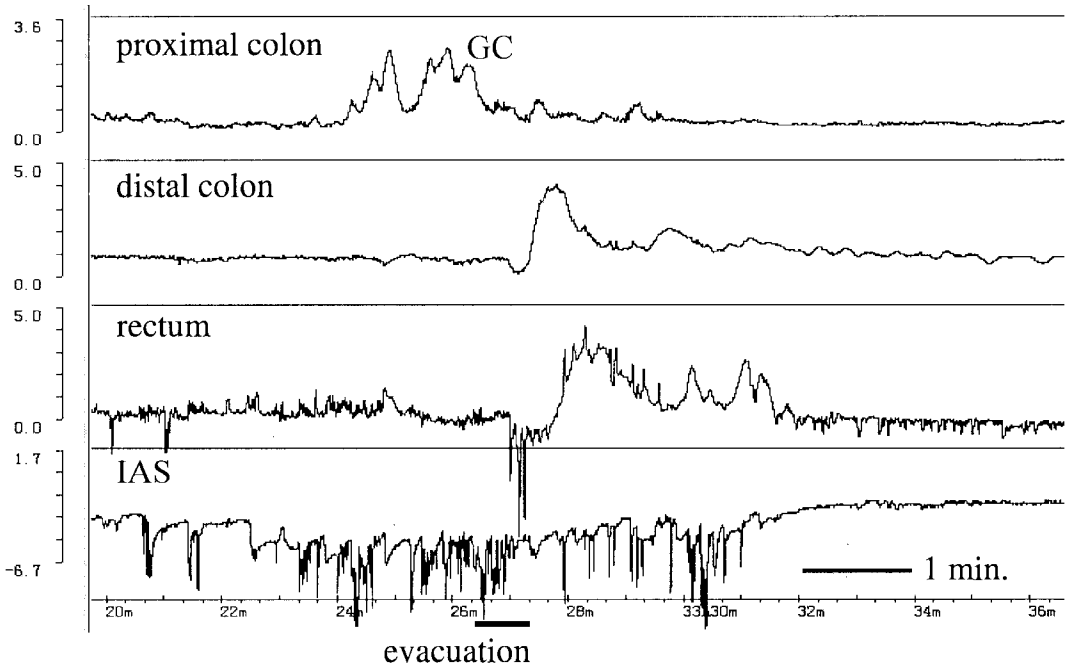

Fig. 3. Mechanical responses of intestinal muscles generated during spontaneous defecation in the dog. Strain gauge force transducers were mounted on the proximal colon, distal colon, rectum and internal anal sphincter (IAS), and mechanical responses of these muscles generated were recorded simultaneously. Evacuation occurred at the bar under the record. GC, giant contraction.

muscles (Fig. 5). The rectum muscles showed a relaxation followed by contraction in two dogs, relaxation alone in two dogs and contraction alone in one dog.

Discusison

The tone of IAS smooth muscle may be myogenic, produced mainly by membrane electrical activities such as slow waves. In many intestinal smooth muscles, spike potentials are the predominant factors to induce contraction. However in IAS smooth muscles, slow waves are capable to induce contraction without the occurrence of the superimposed action potentials (Bouvier and Gonella, 1981). These membrane electrical activities are further regulated by autonomic nerves (Burleigh *et al.*, 1981). The contraction responses of IAS smooth muscles elicited by electrical field stimulation and also produced by exogenously applied norepinephrine are inhibited by phentolamine, indicating that the activity of anal sphincter muscles is controlled largely through α-adrenergic mechanisms (Garett *et al.*, 1974).

The present experiments indicated that the relaxation responses of IAS muscle strips induced by EFS were not modulated by phentolamine, propranolol or atropine. Smooth muscle of the IAS receives non-adrenergic, non-cholinergic (NANC) inhibitory nerves, as in the case of other gastrointestinal tissues, and the neuron, supposed to belong to the enteric neurones, is considered to release nitric oxide, vasoactive intestinal polypeptide (VIP) or adenosine 5'-

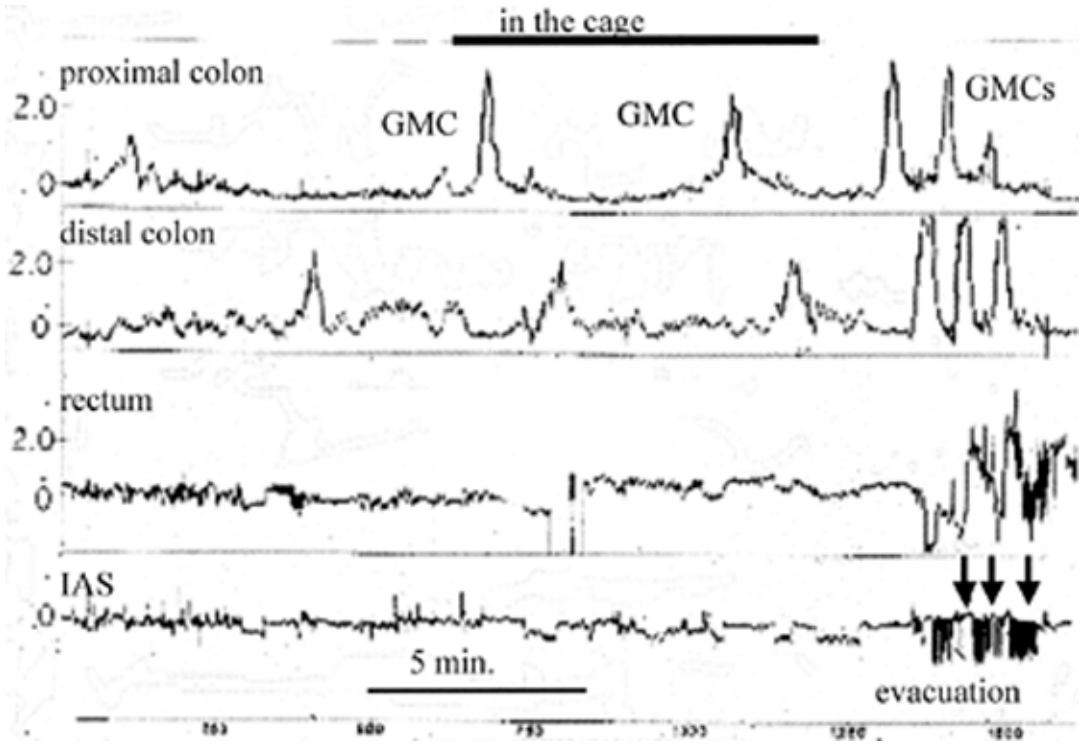

Fig. 4. Mechanical responses of intestinal muscles during defecation in the dog. Responses were recorded simultaneously from the proximal colon, distal colon, rectum and internal anal sphincter (IAS). When the dog was holding to defecate in the cage (shown by bar above the recording), giant migrating contraction (GMC) appeared in the proximal colon. Note that the GMCs did not propagate to the rectum. Three times of evacuation (arrows) were elicited when GMCs propagated to the rectum.

triphosphate (ATP) as a transmitter substance. Although the present experiments did not investigate further the identification of transmitters responsible for the EFS-induced relaxation, possible invelvement of these substances is considered. In patients with Hirschsprung's disease, congenital aganglionosis appears in the most distal part of the colon, inhibitory reflex of IAS is absent (Callaghan and Nixon, 1964). Absence of NANC inhibitory innervation in the IAS of patients with the Hirschsprung's disease is also reported (Matsufuji, 1990). Thus, the NANC inhibitory nerves are taking important physiological roles for the defecation.

In the present study, we recorded the mechanical responses of smooth muscles simultaneously from colon, rectum and IAS, and the aim was to investigate how the activity of IAS muscle was cooperated with that of muscles in the proximal intestine during spontaneous defecation. The characteristic motility patterns of these organs were recognized: defecation was associated with the GCs migrated to the rectum, the relaxation of the rectum preceded the contraction, and the IAS muscle continued to relax while the GCs were migrating. It was revealed that the defecation was associated with the successive phenomena occurring in both the colon and anorectum, and the relaxatory response of IAS muscle during defecation was not merely an extension of the recto-anal inhibitory reflex. These processes are organized primarily

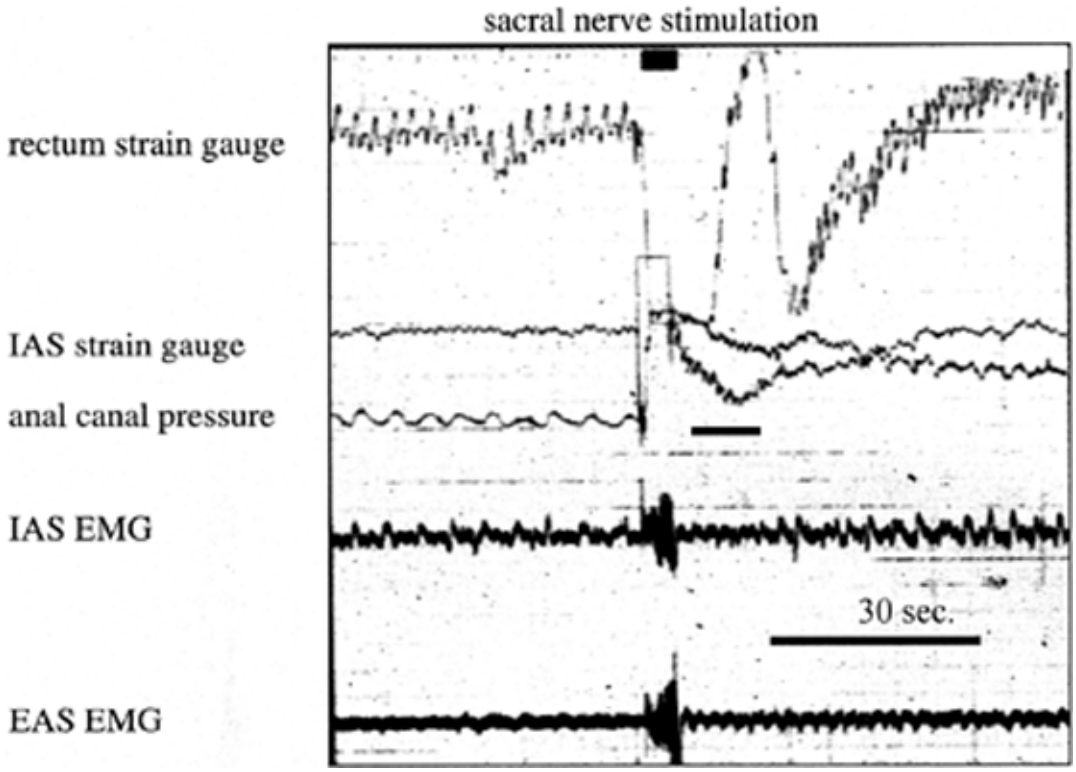

Fig. 5. Mechanical, electrical and pressure responses of anorectum muscles elicited by electrical stimulation of sacral nerves in the dog. Mechanical responses of smooth muscles of the rectum and internal anal sphincter (IAS), anal canal pressure, and electromyogram (EMG) of muscles in the IAS and external anal sphincter (EMS) were recorded simultaneously, while sacral nerves were stimulated (0.5 ms duration, 0.5 mA intensity, 10 Hz frequency) for 5 s (at a bar shown above the recordings). The bar in the figure (under the trace of anal canal pressure) indicates the relaxation of IAS and associated decrease in anal canal pressure, induced by sacral nerve stimulation.

by the enteric nervous systems, as well as the migrating motor complexes of the small intestine or colonic motor complex of the colon (Sarna, 1991). The heuristic model for the enteric nervous system proposed by Wood (1984) indicates that the interneurones connected synaptically into the myenteric nerve networks processes the information from sensory receptors in the intestine and controls the activities of motor neurones. However, this system is not working completely separately, and smooth muscle activities are also under the control of central nervous system *via* the parasympathetic and sympathetic nervous systems.

The parasympathetic nerves originate in the second to fourth sacral nerves and enter the distal colon through pelvic plexus. These nerves ascend orally along the colonic wall in the dog (Fukai and Fukuda, 1984). It is also known that a part of these sacral nerves descends from the rectum to the IAS (Langley and Anderson, 1896a, b, c). We stimulated the sacral nerves electrically, and found that the relaxation followed by contraction of the rectum and the relaxation of IAS muscles, those were observed during spontaneous defecation. Parasympathetic pathway through the sacral nerves may be playing important roles to stimulate

the enteric nervous system and to evoke the synchronized motility of the colon and anorectum in defectaion.

The main sympathetic supply to the colon and anorectum originates from the second to fifth lumber roots. These nerves synapse on the inferior mesenteric ganglia from where the post-synaptic nerves course along the inferior mesenteric artery to synapse the enteric ganglia of the distal colon. The hypogastric nerves also originate from the inferior mesenteric ganglia and distribute to IAS muscles through the pelvic plexus. The injection of α-adrenergic antagonist induced giant migrating contraction associated with defecation in human (Malcom and Camilleri, 2000). The sympathetic nerves may be important for the inhibition of defecation process.

It is concluded that in dogs, the activity of IAS smooth muscle is cooperated with that of smooth muscles in the colon and anorectum, by forming a characteristic motility pattern. The enteric nervous system organizes such coordinated motility and controls the movement of IAS muscles by way of the adrenergic excitatory motor neurones and non-adrenergic, non-cholinergic inhibitory motor neurones. The extrinsic nervous systems might integrate such processes.

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