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Role of the Enteric Nervous System in the Elongated Sigmoid Colon of Patients With Sigmoid Volvulus

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To clarify the physiologic function of the enteric nervous system (ENS) in the elongated sigmoid colon (ESC) of patients with sigmoid volvulus (SV), we examined the enteric nerve responses in lesional and normal longitudinal muscle strips (LMS) derived from patients with ESC and patients who underwent colon resection for colonic cancers. Thirty preparations of LMS were taken from the lesional sigmoid colons of 10 ESC patients with SV (8 men and 2 women, aged 53 to 80 years, mean 66.2 years). Forty preparations of LMS were taken from the normal sigmoid colons (NSC) of 20 patients with colonic cancer (12 men and 8 women, aged 55 to 76 years, mean 62.3 years). A mechanographic technique was used to evaluate in vitro muscle responses to electrical field stimulation (EFS) before and after treatment with various autonomic nerve blockers. Response to EFS before blockade of the adrenergic and cholinergic nerves was as follows: NSC and ESC significantly demonstrated relaxation reaction rather than contraction reaction ($P = 0.0253$, $P < 0.0001$, respectively). ESC showed relaxation reaction more than NSC ($P = 0.1138$). Response to EFS after blockade of the adrenergic and cholinergic nerves was as follows: NSC and ESC significantly demonstrated relaxation reaction via nonadrenergic noncholinergic (NANC) inhibitory nerves rather than contraction reaction via NANC excitatory nerves ($P < 0.0001$, $P < 0.0001$, respectively). ESC with SV significantly showed relaxation reaction more than NSC ($P = 0.0092$). An increased response of relaxation mediated NANC inhibitory nerves may play a role in impaired motility in the ESC of patients with SV.

Key words: Sigmoid volvulus – Elongated sigmoid colon – Enteric nervous system – Nonadrenergic noncholinergic inhibitory nerve – Pathophysiology

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The elongated sigmoid colon (ESC) is considered as a cause of chronic constipation.[1–4] The ESC frequently suffer from sigmoid volvulus (SV).[3,4] In general, ESC in patients with SV is characterized by impaired motility and is associated with changes in the structure of the enteric nervous system (ENS), based on histologic studies.[5–8] However, there are some reports that ENS in the colon of ESC with SV is normal.[9,10] Histopathologic findings in the colon of ESC with SV are still inconclusive.

It is well known that enteric nerves including the nonadrenergic noncholinergic (NANC) excitatory and NANC inhibitory nerves play important roles in the regulation of gut motility.[8,11] Recent studies have suggested that NANC inhibitory nerves act more dominantly than NANC excitatory nerves in the regulation of enteric nerves in the normal colon.[11,12] Mitolo-Chieppa *et al*[12] reported that the ENS is very important for peristalsis of human large intestine and impaired ENS function was found in the colon of patients with slow transit constipation. However, the function of the ENS in the ESC of patients with SV is still unclear. It seems reasonable to infer that physiologic studies of the ENS in the ESC of patients with SV may help to clarify the pathophysiology of impaired motility in patients with ESC.

The ENS attaches to the longitudinal muscle and not circular muscle.[13] In this study, then, we examined the responses of electrical field stimulation (EFS) on both lesional and normal colonic longitudinal muscle strips (LMS) derived from ESC patients with SV and patients who underwent colon resection for colonic cancers by mechanogram.

Patients and Methods

Thirty LMS were taken from the ESC of 10 patients with SV (8 men and 2 women, aged 53 to 80 years, mean 66.2 years) as a control. Forty LMS were taken from the normal sigmoid colons (NSC) of 20 patients with colonic cancer (12 men and 8 women, aged 55 to 76 years, mean 62.3 years).

All ESC patients suffered from chronic constipation with or without abdominal pain for a long period. All ESC patients were also free of secondary causes of constipation by drug history, physical examination, and laboratory screening (blood chemistry, serum electrolytes, thyroid hormones, oral glucose tolerance test, sex hormone profiles, and antinuclear antibodies); and had had no previous abdominal surgery. Organic disease and mechanical obstruction were excluded by barium enema, abdominal ultrasonography, and colonoscopy. Absence of Hirschsprung's disease was documented by normal relaxation of the internal anal sphincter during anorectal manometry. In order to avoid the influence of SV, sigmoidectomy was performed in all ESC patients one month after repositing SV. Histologic sections stained by hematoxylin and eosin were prepared from all regions of each specimen. In histologic studies of the ESC, the complement of nerves and ganglion cells within the myenteric and submucosal plexuses appeared as normal findings. We also found neither ischemic changes nor obstruction/thrombosis in the mesenteric vessels. All control subjects were early sigmoid colon cancers (submucosal cancers without lymph node metastasis) and showed normal defecation before colectomy. Both barium enema and colonoscopy were performed in all control subjects. They were also not accompanied by the other colorectal diseases. The specimens of NSC were also histologically normal and free from invasion by cancer cells.

All ESC patients with SV had undergone sigmoidectomy, and they show normal defecation (stool frequency: 1 time per 1 to 2 days) after operation. They are satisfied with the operation.

Procedure

Both mucosa and circular muscle were removed from the colonic tissues and LMS approximately 1.5 cm in length and 0.5 cm in width were prepared. The materials were placed in a 10-mL organ bath containing Krebs solution (NaCl, 6.9 g/L; KCl, 0.35 g/L; $MgSO_4 \times 7H_2O$, 6.29 g/L; KH_2PO_4, 0.16 g/L; $CaC_{l2} \times 2H_2O$, 0.37 g/L; glucose, 2.0 g/L; $NaHCO_3$, 2.1 g/L: Kantoukagaku, Tokyo, Japan) at 37°C and gassed with 95% O2 and 5% CO2. The movement of the muscle strips was recorded with a pen recorder (Model R-10, Rica Denki, Tokyo, Japan) through an isotonic transducer (Model ME-4012, ME Commercial, Tokyo, Japan) given a 1-g load. The enteric nerves of the muscle strips were electrically stimulated with a clip (Selfin clip, ME Commercial) that was held at both ends and connected to a stimulator (Model ME-6052, ME Commercial). A low frequency of 5 Hz, which is suitable for the stimulation of nerves, with a duration of 0.5 ms, voltage of 50 V, and stimulation time of 30 s was employed.[11] Stimulation was applied according to the square-wave repetitive method. LMS movements were recorded once the muscle strips had become stabilized after 1 hour.

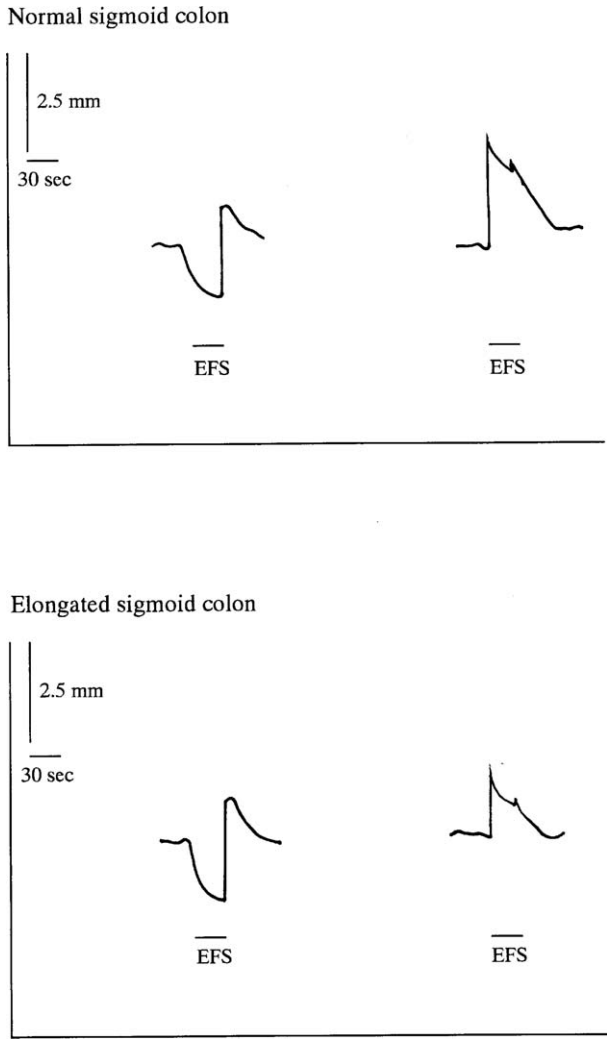

Fig. 1 Response to electrical field stimulation (EFS) before blockade of the adrenergic and cholinergic nerves. The longitudinal muscle strips in both normal sigmoid colon and elongated sigmoid colon demonstrated relaxation rather than contraction. Longitudinal muscle strips of elongated sigmoid colon demonstrated relaxation more than those of normal sigmoid colon.

The following drug preparations were used: atropine sulfate 1×10^{-7} g/ml (Sigma, St. Louis, MO, USA); phenoxybenzamine 5×10^{-6} g/mL (Sigma); propranolol 5×10^{-6} g/mL (Sigma); tetrodotoxin 5×10^{-7} g/mL (Sankyo, Tokyo, Japan).

Statistical analysis

As the observational unit, a preparation was considered rather than a patient, because from 1 to 2 muscle strips per patient were used (ESC: 1 or 2 muscle strips per patient; NSC: 2 muscle strips per patient). The Chi-squared test (two-tailed) was used. Statistical analysis was carried out with StatView (version 5.0 for Windows; Abacus Concepts Inc, Berkeley, CA) and a value of $P < 0.05$ was considered significant.

Table 1 Response to electrical field stimulation before blockade of the adrenergic and cholinergic nerves

	Contraction	Relaxation
Normal sigmoid colon	37.5% (15/40)[a]	62.5% (25/40)[b]
Elongated sigmoid colon	20.0% (6/30)[c]	80.0% (24/30)[d]

a versus b: $P = 0.0253$, c versus d: $P < 0.0001$, b versus d: $P = 0.1138$.

Results

Experiment 1

To determine whether the NANC excitatory and inhibitory nerves were present in the NSC and in the ESC of patients with SV, the responses to EFS before and after blockade of the adrenergic and cholinergic nerves were studied.

Response to EFS before blockade of the adrenergic and cholinergic nerves

Fig. 1 illustrates the results of a typical experiment. A contraction reaction (right side) and a relaxation reaction (left side) were respectively seen in 37.5% and 62.5% of the NSC muscle strips. These reactions were respectively seen in 20.0% and 80.0% of the ESC muscle strips. NSC and ESC muscle strips significantly demonstrated relaxation reaction rather than contraction reaction ($P = 0.0253$, $P < 0.0001$, respectively). In addition, ESC muscle strips showed relaxation reaction more than NSC muscle strips ($P = 0.1138$; Table 1).

Response to EFS after blockade of the adrenergic and cholinergic nerves

Fig. 2 illustrates the results of a typical experiment. A contraction reaction (right side) and relaxation reaction (left side) were respectively seen in 20.0% and 80.0% of the NSC muscle strips. These reactions were respectively seen in 0% and 100% of the ESC muscle strips. NSC and ESC muscle strips significantly demonstrated relaxation reaction rather than contraction reaction ($P < 0.0001$, $P < 0.0001$, respectively). In addition, ESC muscle strips significantly showed relaxation reaction more than NSC muscle strips ($p = 0.0092$; Table 2).

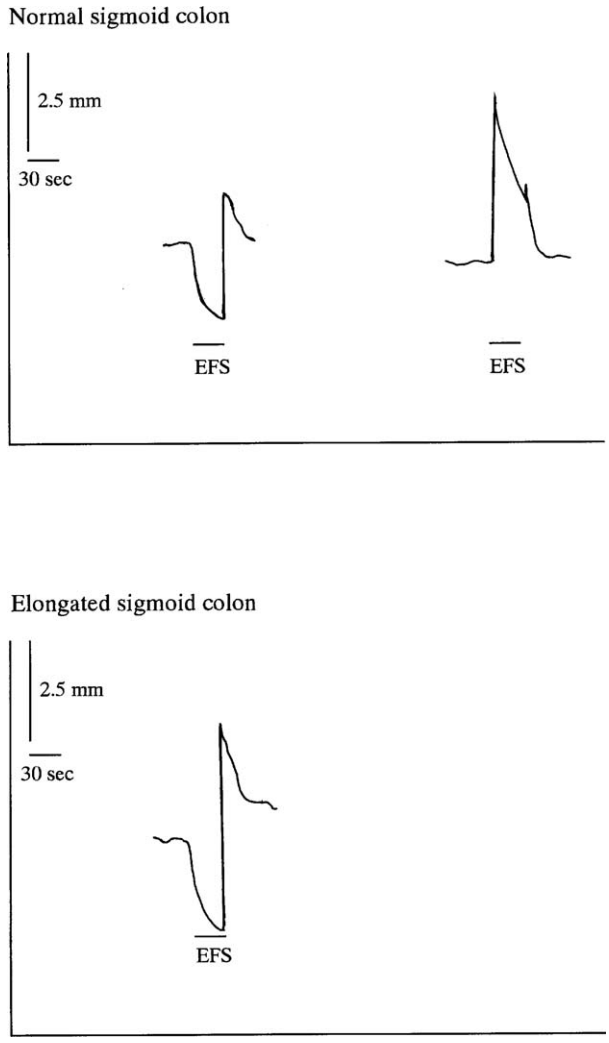

Fig. 2 Response to electrical field stimulation (EFS) after blockade of the adrenergic and cholinergic nerves. The longitudinal muscle strips in normal sigmoid colon demonstrated relaxation rather than contraction. All longitudinal muscle strips of elongated sigmoid colon demonstrated relaxation.

NANC excitatory and inhibitory nerves were found to act on the muscle strips of NSC and ESC. NANC inhibitory nerves in ESC were more involved in the regulation of enteric nerves than those in the NSC.

Experiment 2

It was uncertain whether responses to EFS acted via the nerves or by a direct effect on the smooth muscle. To clarify this point, the effects of EFS after blocking the nerves with tetrodotoxin (a well-known neurotoxin) were studied.

Table 2 Response to electrical field stimulation after blockade of the adrenergic and cholinergic nerves

	Contraction	Relaxation
Normal sigmoid colon	20.0% (8/40)[a]	80.0% (32/40)[b]
Elongated sigmoid colon	0% (0/30)[c]	100.0% (24/30)[d]

a versus b: $P < 0.0001$, c versus d: $P < 0.0001$, b versus d: $P = 0.0092$.

Following addition of tetrodotoxin after blockade of the adrenergic and cholinergic nerves, the responses to EFS were recorded. Tetrodotoxin completely abolished the EFS responses in the muscle strips of both NSC and ESC muscle strips (Fig. 3, Table 3). These results suggested that EFS alone stimulated the ENS in the NSC and ESC muscle strips.

According to these results, an increased response of relaxation mediated inhibitory nerves, especially NANC inhibitory nerves, was observed in the ESC of patients with SV compared with NSC.

Discussion

The ESC associates with a long and narrow mesocolon at its parietal attachment, resulting in the two ends of the loop being close together, under which conditions the sigmoid colon twists around its central mesenteric axis. The clinical symptoms are chronic constipation with or without abdominal pain and/or abdominal distension before the development of SV.[10] In surgery, SV is most common in patients with ESC. Many patients with ESC have clinical findings indicating impaired colonic movement.[15] Clinically, dilatation and elongation of the sigmoid colon are seen in patients with ESC and the ESC exhibits a reduction in contractile activity in the sigmoid colon on colonoscopy.[14,15] It has been suspected that pathologic changes in the ENS could be involved in the pathophysiology of ESC.[5–7,16] Collure *et al*[16] reported that the decrease and loss of ganglion cells are only a consequence, not a cause, since the exact cause of ESC is not clear. In our histologic studies of the ESC, the number of intramural ganglion cell was normal. However, the function of the ENS in patients with ESC is still poorly understood.

Recently, it has been considered that NANC nerves are more important to the ENS than cholinergic and adrenergic nerves.[9,15,17] The intestinal peristaltic reflex is mediated via the ENS, and NANC excitatory nerves have been found to bring about proximal side contraction while NANC

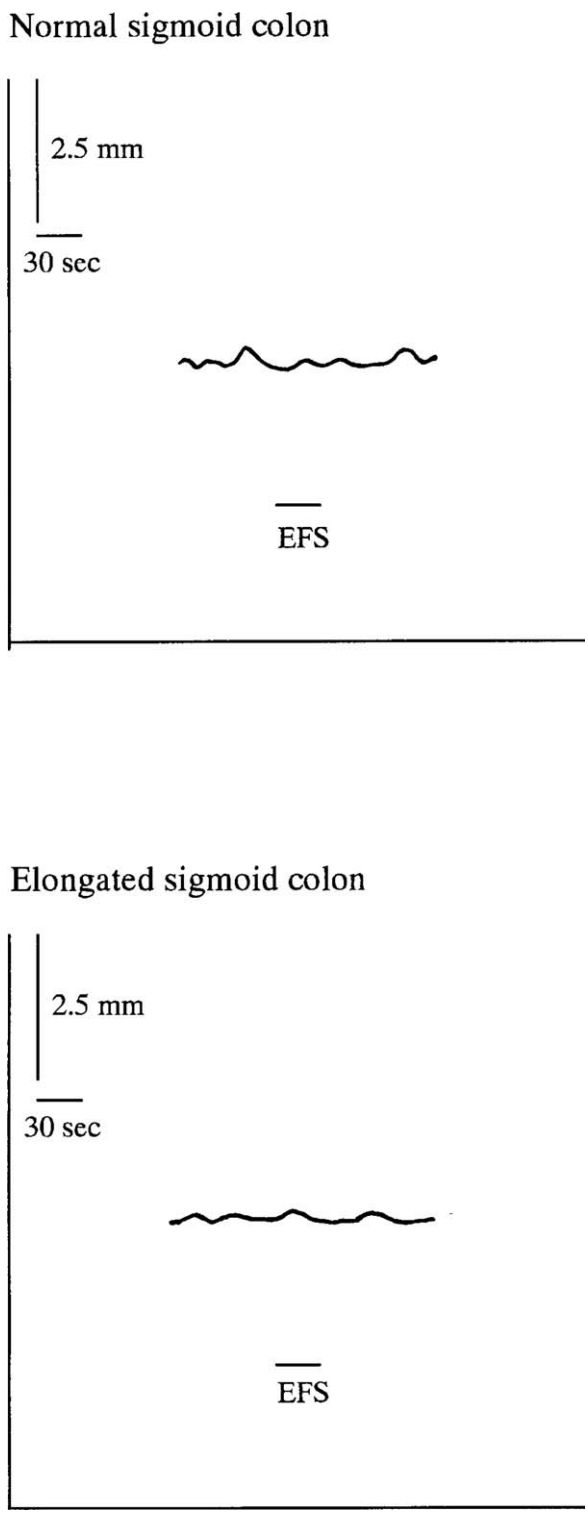

Fig. 3 Response to electrical field stimulation (EFS) after administration of tetrodotoxin following blockade of the adrenergic and cholinergic nerves. No reactions to EFS were seen in the longitudinal muscle strips of normal sigmoid colon and elongated sigmoid colon.

Table 3 Response to electrical field stimulation after administration of tetrodotoxin following blockade of the adrenergic and cholinergic nerves

	No reaction
Normal sigmoid colon	100% (40/40)
Elongated sigmoid colon	100% (30/30)

inhibitory nerves cause distal side relaxation of the bowel.[17] Previous reports suggest that the innervation contains both NANC excitatory and inhibitory nerves and that the latter are dominant.[11,17] However, the role of the NANC excitatory and inhibitory nerves in the ESC remains unclear. In the present studies, NANC excitatory and inhibitory nerves were found to act on both NSC and ESC. NANC inhibitory nerves in ESC were more significantly involved in regulation of enteric nerves than those in the NSC. These results showed that an increased response of relaxation-mediated NANC inhibitory nerves play an important role in the reduced motility observed in the ESC of patients with SV. Thus, the ESC patients undergoing sigmoidectomy for SV are free from disorder of bowel movement such as chronic constipation with or without abdominal pain and/or abdominal distension.[10,15] However, some researchers have claimed that patients undergoing sigmoidectomy for SV suffer from elongation and dilatation of the remaining colon, and some even require surgery for revolvulus.[18] Some authors have claimed that SV might be one of the disorders allied with Hirschsprung's disease.[19,20] In this study, functional disorder of bowel movement or dilatation of the colon in ESC with SV is not found with the histologic abnormalities. Therefore, an impaired motility of the ESC may be caused by dysfunction of the NANC inhibitory nerves. All ESC patients with SV showed normal bowel habit (stool evacuation: once per 1 to 2 days) less than 1 month after sigmoidectomy and satisfied with postoperative status. Because, they are free from constipation with abdominal pain and/or abdominal distension. We showed that dysfunction of the NANC inhibitory nerves may have more of an effect on relaxation seen in the ESC. If this theory is correct, one would assume that even after the surgery for volvulus, the problem remains, as the remaining part of the bowel would be under the influence of the same nerves, and there would be a chance of recurrence. ESC patients with SV are less than 1 year after sigmoidectomy. We cannot say for certain whether a chance of re-volvulus will occur or

not in a long period postoperation. Therefore, we are carefully following these patients.

In conclusion, this study supports the hypothesis that the intrinsic NANC inhibitory neuronal input to smooth muscle is increased in the ESC with SV. This may be related to the pathophysiology of ESC colonic disorder. Incidentally, it has been reported that patients with slow transit constipation have specific abnormalities of circulating gut hormones such as serotonin, somatostatin, pancreatic glucagon, enteroglucagon, gastric inhibitory peptide, peptide YY, pancreatic polypeptide, motilin, serotonin, cholecystokinin, and NT.[21–23] Various kinds of neuropeptides, nitric oxide, cytokines, and interstitial cells of Cajal have recently been shown to work together in the ENS.[6] These act mutually, and an overall evaluation of these substances needs to be made in the future.

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