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ORIGINAL PAPER

Alteration of neuromuscular transmissions in the hamster colon following the resolution of TNBS-induced colitis

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Abstract The aim of this study was to determine whether trinitrobenzene sulfonic acid-induced colitis leads to alterations in enteric neuronal transmission in hamsters. We assessed the mechanical responses induced by the application of electrical field stimulation (EFS) in isolated segments of the distal colon. The EFS-induced relaxation and contraction were blocked by a nitric oxide synthase inhibitor and by the combination of antagonists for tachykinin NK_1 and NK_2 receptors and muscarinic acetylcholine receptors, respectively. The mechanical responses to EFS were attenuated in the inflamed colon at 7 days and were recovered by 30 days after inflammation treatment. In addition, we found that purinergic and opioidergic excitatory neural components are expressed following the resolution of colitis. These results suggest that colonic inflammation causes indiscriminate damage to enteric neurons but that neuronal components are restored and that new excitatory neural components, compensating for the contractile responses in smooth muscle after colitis, are expressed.

Keywords Colitis · Enteric nervous system · Gastrointestinal motility · Neuronal alteration · TNBS

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Introduction

The enteric nervous system (ENS) plays an essential role in the regulation of gastrointestinal function [1–3]. The ENS contains intrinsic primary afferent neurons, interneurons and motor neurons, and these components form reflex arcs to coordinate gastrointestinal motility [1]. Myenteric motor neurons release excitatory and inhibitory neurotransmitters for the contraction and relaxation of intestinal smooth muscle, respectively [3, 4]. Peristalsis is characterized by ascending contraction and descending relaxation [1, 3]. It has been shown that nonadrenergic noncholinergic (NANC) neurotransmitters released by inhibitory neurons, including nitric oxide (NO), ATP and vasoactive intestinal peptide (VIP), mediate descending relaxation [3–5], while ascending contraction is brought about by the release of acetylcholine and tachykinins from excitatory motor neurons [3–5].

During inflammatory processes, this regulation by the ENS is disrupted, as has been shown in human inflammatory bowel disease (IBD) and in animal models of IBD [6–11]. Chronic inflammation of the intestine is not restricted to the mucosa but extends to the submucosa and muscularis externa, thus influencing neurotransmission and muscle contractility [12]. Functional alterations of adrenergic, cholinergic, purinergic and nitrergic neurotransmission have been reported in the inflamed intestine [6, 13, 14]. In addition, persistent alterations in gastrointestinal function are commonly observed after the resolution of intestinal inflammation [8, 15–19]. These include changes in visceral sensation, abnormal secretion and altered motility patterns.

It is possible that persistent alterations in neuronal transmission following a bout of inflammation may contribute to the long-term functional consequences of prior inflammation. However, how prior inflammation affects the

subpopulation of enteric neurons is not clear. Therefore, the aim of our study was to determine whether trinitrobenzene sulfonic acid (TNBS)-induced colitis leads to alterations in enteric neuronal transmission and peristaltic motility that persist beyond the resolution of inflammation. In particular, we focused on expression of alternative purinergic and opioidergic components at the post-inflammatory stage because previous reports indicate that changes in these components of enteric neurons are facilitated and/or attenuated in the inflamed colon of animal models and IBD patients [20–26]. To this end, constant induction of colitis is required since the seriousness of inflammation cannot be judged after resolution. In our previous experiments, we found that the hamster is a valuable animal model of inducible colitis with TNBS which produces consistent and reproducible results [27]. In addition, NANC neurotransmissions in the hamster colon have been reported [28, 29]. We therefore used hamsters in the study reported here.

Methods

Animals

Male Syrian hamsters, 8–10 weeks of age, were obtained from Japan SLC (Shizuoka, Japan). These were maintained in plastic cages at 22 ± 2 °C under a 12-h light/dark cycle and given free access to laboratory chow (LABO MR Stock, Nihon-Nosan, Yokohama, Japan) and water. The experiments were approved by the Gifu University Animal Care and Use Committee (permission numbers: 09016, 10007 and 11126) and were conducted in accordance with the committee guidelines on animal care and use.

Induction of colitis

Colitis was induced according to a method described by Morris et al. [30] with slight modifications. After overnight fasting, animals were anaesthetized with pentobarbitone (50 mg/kg, intraperitoneal). They were then given 10 mg of TNBS dissolved in 0.25 ml of 40 % ethanol (v/v) by means of a silicon catheter inserted 5 cm through the anus. The animals were maintained in a head-down position for 10 min to prevent leakage of the intracolonic instillate. Hamsters in the control group were given an enema of the same volume of 0.9 % saline instead of the TNBS solution. All hamsters were allowed to remain in their home cages until sacrificed at 7 or 30 days after TNBS or saline administration.

Tissue preparation

Colonic segments for mechanical recordings and measurement of peristaltic movement were prepared as previously described [28]. Animals were anesthetized using isoflurane and exsanguinated via the carotid artery. The abdominal cavity was opened immediately, and a 3- to 4-cm-long segment of the distal colon (1.5 cm from the anus) was dissected out and immersed in physiological salt solution (PSS; see below) at room temperature. The intraluminal contents were flushed using a small cannula filled with PSS. Each segment was used only once for either the mechanical recording or measurement of peristaltic movement.

Mechanical recordings

A 2- to 3-cm-long segment of the distal colon was mounted in an organ bath (capacity 10 ml) filled with PSS (pH 7.4). The solution was continuously bubbled with a 95 % O_2 + 5 % CO_2 gas mixture and maintained at 37 °C. The distal end of each segment was tied to organ holders, and the proximal end was secured with a silk thread to an isometric force transducer. The preparation was stimulated electrically by means of two platinum electrodes, one of which was placed in the lumen of the preparation and the other in the bathing solution (coaxial stimulation). Supramaximal rectangular pulses of 20 V in intensity and 0.3 ms in duration were delivered by using an electrical stimulator (model SEN-3301; Nihon Kohden, Tokyo, Japan) with frequency spectra of 2 or 20 Hz for 1 s. Contractile activity was recorded isometrically with a force transducer (T7-30-240; Orientec, Tokyo, Japan). An initial tension of 1.5 g was applied to the colonic preparations, which were subsequently allowed to equilibrate for 45–60 min. At the end of this period, the tension created by the segment was considered to be the resting tension, and no further mechanical adjustment was made during experimentation. Isometric responses were filtered and amplified by an amplifier (model AS1202; NEC Corp., Tokyo, Japan) and recorded using a PowerLab system (AD Instruments, Bella Vista, NSW, Australia).

Measurement of peristaltic movement

The Trendelenburg method [31] was used for recording peristaltic activity in response to intraluminal distension. Briefly, the colonic segment (length 5 cm) was positioned in a 100-ml organ bath filled with PSS (pH 7.4). The solution was continuously bubbled with the 95 % O_2 + 5 % CO_2 gas mixture and maintained at 37 °C. The ligated oral end of the colonic segment was attached to an isotonic transducer (model IT-10; Medical Agent, Japan), and the anal end of the segment was tied around the mouth of a U-shaped glass tube connected to the outflow line. A pressure transducer (MP5100; Baxter, Tokyo, Japan) and a bottle of PSS for pressure stimulation were connected to

the outflow line via a T-connector. Changes in intraluminal pressure were recorded with a chart recorder (RTA-1000; Nihon Kohden, Tokyo, Japan). The peristaltic reflex of the isolated colon was initiated by increasing the intraluminal pressure to apply distention. The pressure stimulation was applied by gradually raising the height of the bottle containing the PSS that was connected to the anal end of the colon (about 1 cm/s). The strength of the pressure against the intestinal wall was represented as liquid pressure (cmH_2O), which was equivalent to the height between the level of liquid within the bottle connected to the anal end of the colon and the preparation.

Macroscopic evaluation of the inflamed colon

The distal colon was removed, longitudinally opened, gently cleaned of fecal content and then spread to observe the luminal surface. The severity of macroscopically visible colonic damage was scored using the scoring system described in Table 1, which was adapted from that used previously [9]. This system took into consideration the absence/presence of hyperemia, the area of necrosis and ulcers and the absence/presence of adhesion between the colon and other organs.

Solutions and drugs

During the experiments, tissues were maintained in PSS consisting of (in mM): NaCl, 118.4; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 25; glucose, 11.7 (modified Krebs' solution). Tetrodotoxin was used to block the voltage-dependent sodium channels on neurons, atropine was used to block the muscarinic acetylcholine receptors on smooth muscle cells and *N*-acetyl-L-tryptophan 3,5-bis (trifuluorometyl) benzyl ester (L-732,138) and (S)-*N*-methyl-*N*[4-(4-acetylamino-4-phenylpiperidino)-2-(3,4-dichlorophenyl) butyl] benzamide (SR48968) were used to block tachykinin NK_1 and tachykinin NK_2 receptors, respectively. N_ω-Nitro-L-arginine methyl ester hydrochloride (L-NAME) was used as an NO synthase (NOS) inhibitor, and 1H-[1,2,4]Oxadiazolo[4,3-a]quinoxalin-1-one (ODQ) was used as an inhibitor of soluble guanylyl cyclase, resulting in competition with NO. Suramin was used to block purinocetors, and naloxone was used to block opioid receptors. L-732,138, L-NAME, naloxone, ODQ and suramin were obtained from Sigma (St. Louis, MO). Atropine sulfate salt monohydrate, TNBS and tetrodotoxin were obtained from Wako (Osaka, Japan) and SR48968 was a gift from Sanofi-Synthelabo (Montpellier, France). L-732,138 and SR48968 were dissolved in ethyl acetate and ethanol, respectively. The vehicles (ethanol and ethyl acetate) for the drugs alone had no effect on basal tone. Other drugs were dissolved in distilled water. The drug concentrations given in the text were final concentrations in the bath solution.

Table 1 Criteria for scoring of gross morphology

Feature graded	Grade	Description
Hyperemia	0	None
	1	Mild
	2	Severe
Ulcer/necrosis	0	None
	1	Damage: <1 cm length
	2	Damage: 1–2 cm length
	3	Damage: 2–3 cm length
	4	Damage: >3 cm length
Adhesion	0	None
	1	Presence

Statistical analysis

Data are presented as the mean ± standard deviation, and n indicates the number of experiments performed using different tissue preparations from different hamsters. The significance of differences between mean values was determined by one-way analysis of variance followed by Dunnet's test for comparison of multiple groups and by Student's or paired t test for comparison of two groups. A p value of <0.05 denotes the presence of a statistically significant difference.

Results

EFS-induced mechanical responses in the normal colon

In segments of the distal colon from normal hamsters, EFS (2 Hz) evoked biphasic mechanical responses consisting of initial fast relaxation followed by contractions (Fig. 1a). The first relaxation component reached a peak value about 3.6 ± 0.3 s after stimulation, and the second contraction component reached a peak value about 7.7 ± 0.1 s after stimulation. A higher EFS frequency (20 Hz) induced contractile responses but not relaxation in the hamster colon (Fig. 1b). Both contractile and relaxation responses were abolished by the application of tetrodotoxin (1 μM), a blocker of voltage-dependent sodium channels on neurons ($n = 5$, data not shown).

Effects of L-NAME and ODQ on EFS-evoked relaxation responses

To examine whether NO mediates EFS (2 Hz)-induced relaxation in the hamster colon, L-NAME (50 or 200 μM),

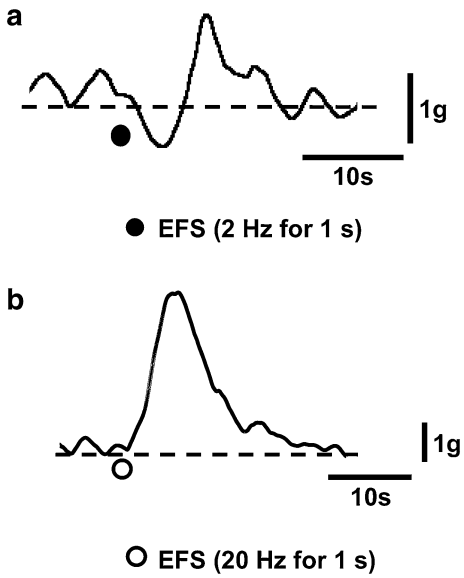

Fig. 1 Mechanical responses elicited by electrical field stimulation (EFS) in an isolated segment of the hamster colon. Representative tracings of EFS-mediated contraction and relaxation in the hamster colon are shown. Electrical stimulation was applied using multi-pulses of 20 V each, with 0.3-ms pulse duration at 2 Hz (**a**) or 20 Hz (**b**) for 1 s; longitudinal mechanical responses were recorded isometrically. *Circles* Points of electrical stimulations

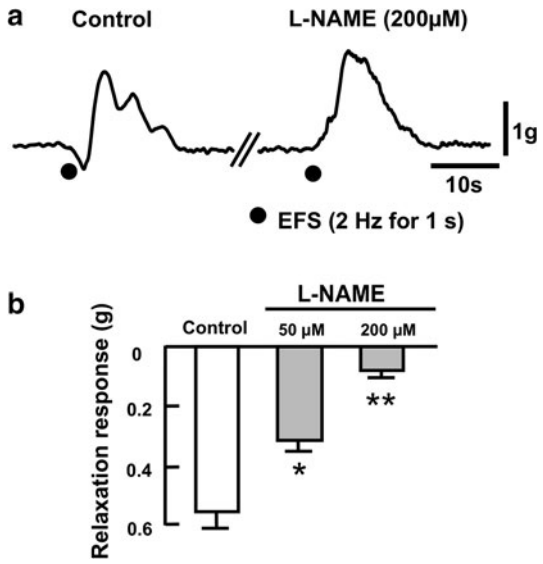

Fig. 2 Effects of a nitric oxide synthase (NOS) inhibitor on relaxation responses elicited by EFS in the hamster colon. **a** A representative tracing of EFS-mediated response in the hamster colon in the presence or absence of N_{ω}-nitro-L-arginine methyl ester hydrochloride (*L-NAME*) (200 μM), an NOS inhibitor, is shown. EFS was applied using multi-pulses of 20 V each, with a 0.3-ms pulse duration at 2 Hz for 1 s; longitudinal mechanical responses were recorded isometrically. *Circles* Points of electrical stimulations. **b** Summary graphs of the effects of L-NAME (50 or 200 μM) on EFS-evoked relaxation in the hamster colon ($n = 6$). Each value represents the mean ± SD. $^{**}p < 0.01$ and $^{*}p < 0.05$ compared to the control, which is the response before the addition of L-NAME

an NOS inhibitor, was applied to the preparation 30 min prior to EFS. As shown in Fig. 2, L-NAME application reduced EFS-induced relaxation in a dose-dependent manner. ODQ (10 μM), a soluble guanylate cyclase inhibitor, also reduced the relaxation response significantly when it was applied 30 min before EFS ($n = 5$; $p < 0.05$; data not shown).

Effects of tachykinin receptor antagonists and atropine on contractile response

We next examined the neural components responsible for contractile responses. To simplify the interpretation of results of pharmacological experiments, blockers were applied under the condition in which the relaxation component of EFS (2 Hz)-induced responses was eliminated by L-NAME (200 μM). Pretreatment with blockers for tachykinin NK_1 receptors, tachykinin NK_2 receptors or muscarinic cholinoceptors (L-732,132, SR48968 and atropine, 1 μM, respectively) significantly inhibited the EFS-evoked contractile responses (Fig. 3), while the combined application of blockers for tachykinin receptors (1 μM) significantly diminished the EFS-evoked contractile responses (Fig. 3). In addition, the contraction, which was resistant to tachykininergic antagonists, was almost totally blocked by the cumulative application of atropine. EFS (20 Hz)-elicited contractile responses were also blocked by the application of blockers for tachykinin NK_1 and tachykinin NK_2 receptors (1 μM) and atropine (1 μM) (Fig. 4).

Effects of inflammation on EFS-induced contractile and relaxation responses

Colitis was induced by injecting TNBS into the distal colon. Diarrhea occurred 3–7 days after the TNBS treatment. Seven days after treatment with TNBS, the body weight of hamsters had decreased to 83 ± 2.5 g from an initial weight of 97 ± 3.4 g ($n = 10$). In contrast, the weight of control animals increased by approximately 10 g over the 7-day period, from 95 ± 2.3 to 105 ± 3.1 g ($n = 6$). Macroscopic observation showed that treatment with TNBS induced visible inflammation to the colon, as indicated by the macroscopic damage score, 4.5 ± 0.4 ($n = 5$), at 7 days post-TNBS treatment. The colon of hamsters at 7 days after TNBS injection contained diarrheal feces. In contrast to the intact colon, EFS (2 Hz) did not evoke mechanical responses in the isolated inflamed colon at 7 days after TNBS treatment (Fig. 5a). Increment of the stimulus frequency up to 20 Hz induced contractile responses in the inflamed colon, although the maximal contraction amplitudes were less than those observed in the normal colon (Fig. 5b). The contractile

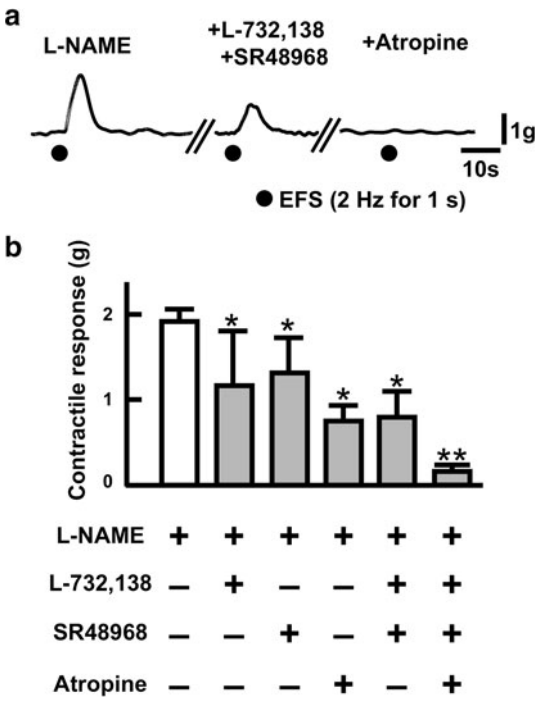

Fig. 3 Effects of tachykininergic and cholinergic blockers on contractile responses elicited by low-frequency EFS in the hamster colon. **a** A representative tracing showing the effects of L-732,138 (1 μM) and SR48968 (1 μM), blockers for tachykinin NK_1 and tachykinin NK_2 receptors and atropine (1 μM), an antagonist of muscarinic acetylcholine receptors, on EFS-induced contraction in the hamster colon in the presence of L-NAME (200 μM). EFS was applied using multi-pulses of 20 V each, with a 0.3-ms pulse duration at 2 Hz for 1 s; and longitudinal mechanical responses were recorded isometrically. *Circles* Points of electrical stimulations. **b** Summary graphs of the effects of L-732,138 (1 μM), SR48968 (1 μM) and atropine (1 μM) on EFS-evoked contraction in the hamster colon in the presence of L-NAME (200 μM) ($n = 6$). Each value represents the mean ± standard deviation (SD). $^{**}p < 0.01$ and $^{*}p < 0.05$ compared to the control, which is the response before the addition of blockers

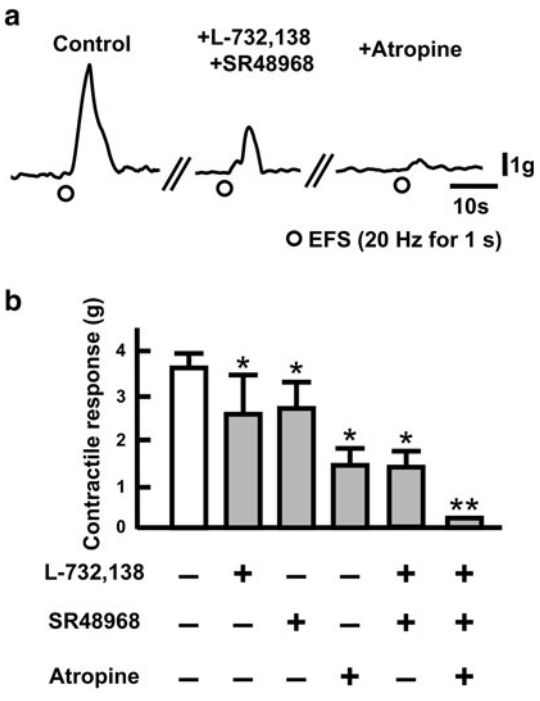

Fig. 4 Effects of tachykininergic and cholinergic blockers on contractile responses elicited by high-frequency EFS in the hamster colon. **a** A representative tracing showing the effects of L-732,138 (1 μM) and SR48968 (1 μM), blockers for tachykinin NK_1 and tachykinin NK_2 receptors, and atropine (1 μM), an antagonist of muscarinic acetylcholine receptors, on EFS-induced contraction in the hamster colon. EFS was applied using multi-pulses of 20 V each, with a 0.3-ms pulse duration at 20 Hz for 1 s; longitudinal mechanical responses were recorded isometrically. *Circles* Points of electrical stimulations. **b** Summary graphs of the effects of L-732,138 (1 μM), SR48968 (1 μM), and atropine (1 μM) on EFS-evoked contraction in the hamster colon ($n = 6$). Each value represents the mean ± SD. $^{**}p < 0.01$ and $^{*}p < 0.05$ compared to the control, which is the response before addition of blockers

responses were blocked by the application of blockers for tachykinin NK_1 and tachykinin NK_2 receptors (1 μM) and atropine (1 μM) (data not shown).

To determine whether the diminished mechanical response observed after treatment with TNBS is reversible or not, hamsters injected with TNBS were kept alive for 30 days. The diarrhea induced by TNBS was not observed at 30 days post-TNBS treatment. The body weight of hamsters at 30 days after TNBS injection was 123 ± 3.4 g ($n = 5$), which was comparable to that of the control animals, 125 ± 2.1 g ($n = 6$). The colonic damage score of the hamsters at 30 days after TNBS treatment was below the detection level, and their colon contained well-formed solid feces as in control hamsters. As shown in Fig. 5, mechanical responses, which were comparable to those of controls, were induced by the application of EFS in the colon at 30 days after TNBS treatment.

Pharmacological analysis of EFS-induced responses in the colon at 30 days after TNBS treatment

We then examined whether the recovery of responses is attributable to the recovery of neural components that operate under normal conditions or whether it is related to compensatory mechanisms. Application of L-NAME (200 μM) significantly reduced EFS (2 Hz)-evoked relaxation in the colon isolated 30 days after TNBS treatment (Fig. 6a). In addition, the application of blockers for tachykinin NK_1 and tachykinin NK_2 receptors (1 μM) and atropine (1 μM) inhibited the contractile response elicited by EFS (2 Hz), which was recorded in the presence of L-NAME (200 μM) (Fig. 6b).

In contrast, EFS (20 Hz)-induced contractile response was not blocked by the addition of either tachykinin receptor blockers (1 μM) or atropine (1 μM) (Fig. 7). The non-cholinergic/non-tachykininergic response was significantly attenuated by antagonists of purinocetors and opioid

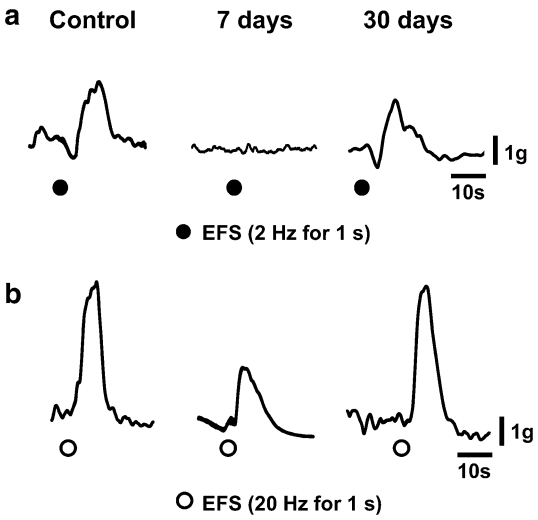

Fig. 5 Effects of inflammation on mechanical responses elicited by EFS in an isolated segment of the hamster colon. Representative tracings of EFS-mediated contraction and relaxation in intact and inflamed hamster colons at 7 or 30 days after trinitrobenzene sulfonic acid (TNBS) treatment are shown. Electrical stimulation was applied using multi-pulses of 20 V each, with a 0.3-ms pulse duration at 2 Hz (**a**) or 20 Hz (**b**) for 1 s; longitudinal mechanical responses were recorded isometrically. *Circles* Points of electrical stimulations

receptors (suramin and naloxone, 400 and 100 μM, respectively) (Fig. 7). The contraction induced by EFS in the presence of tachykininergic, cholinergic, purinergic and opioidergic antagonists was completely blocked by tetrodotoxin (1 μM), an inhibitor of neurogenic responses (Fig. 7). In contrast, the application of tetrodotoxin increased/enhanced spontaneous movements in the colonic segment (Fig. 7a).

Effects of inflammation on peristaltic responses in the colon

Figure 8 shows a typical trace of peristaltic movements in the intact and inflamed hamster colons. Increments of intraluminal pressure up to 2 cmH_2O induced propulsive peristaltic response. Relaxation and contraction responses in longitudinal muscle represent the preparatory phase of peristalsis (Fig. 8a), and intraluminal pressure changes represent the empty phase of peristalsis (Fig. 8b). Peristaltic waves with a frequency of 2.0 ± 0.1 times/5 min were generated in the segment of the distal colon by application of 2 cmH_2O luminal pressure in the normal colon. These responses were blocked by exposure to tetrodotoxin (1 μM) ($n = 4$, data not shown). In contrast, the peristaltic responses were not induced or only weakly induced even after the increase in intraluminal pressure in the inflamed colon at 7 days after TNBS treatment (Fig. 8). At 30 days after TNBS treatment, the propulsive peristaltic response was recovered (Fig. 8).

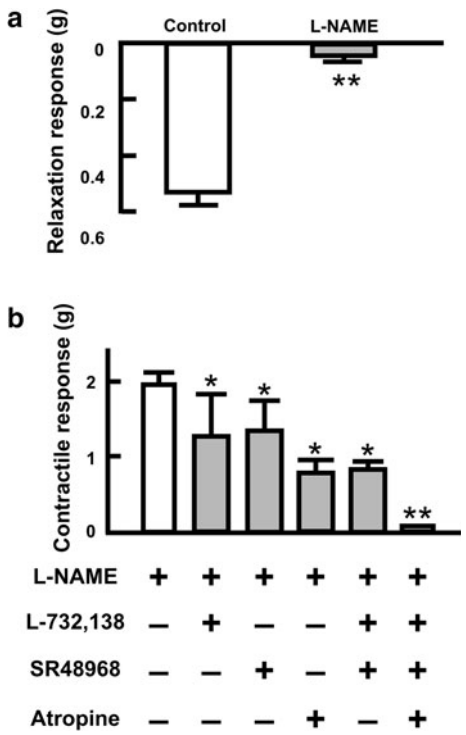

Fig. 6 Effect of an NOS inhibitor on relaxation responses and effects of tachykininergic and cholinergic blockers on contractile responses elicited by low-frequency EFS in the hamster colon at 30 days after TNBS treatment. Summary graphs of the effect of L-NAME (200 μM) on EFS-evoked relaxation (**a**) and effects of L-732,138 (1 μM), SR48968 (1 μM) and atropine (1 μM) on EFS-induced contraction in the presence of L-NAME (200 μM) (**b**) in the post-inflamed hamster colon ($n = 5$). EFS was applied using multi-pulses of 20 V each, with a 0.3-ms pulse duration at 2 Hz for 1 s; longitudinal mechanical responses were recorded isometrically. Each value represents the mean ± SD. $^{**}p < 0.01$ and $^{*}p < 0.05$ compared to the control, which is the response before addition of blockers

Discussion

Electrical stimulation of the enteric nerves elicited an initial relaxation followed by a contraction response in the hamster distal colon. NO seems to be the most general mediator of relaxation in of the gastrointestinal tract [3, 4]. In line with this, EFS-induced relaxation was completely blocked by a NOS blocker and by a soluble guanylate cyclase inhibitor, indicating that NO is the main transmitter for the relaxation response. On the other hand, combined application of NK_1 and NK_2 tachykinin receptor antagonists and a muscarinic antagonist abolished the EFS-induced contractile response. This finding indicates that tachykininergic and cholinergic neurotransmitters are responsible for the contractile response. This is supported by the results of a study by Maggi and Giuliani [32] showing that NANC-induced contraction of the circular muscle of the rat small intestine in response to EFS involves tachykinins, acting via tachykinin NK_1 and NK_2

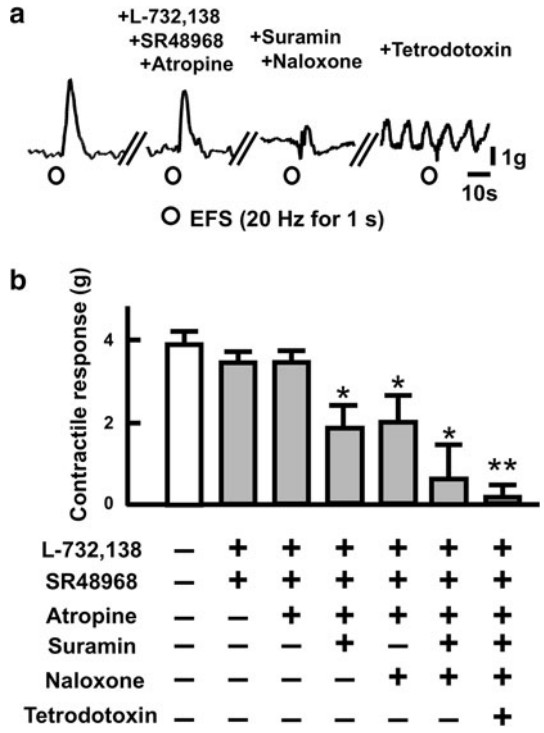

Fig. 7 Effects of tachykininergic and cholinergic blockers on contractile responses elicited by high-frequency EFS in the hamster colon at 30 days after TNBS treatment. **a** A representative tracing showing the effects of L-732,138 (1 μM) and SR48968 (1 μM), blockers for tachykinin NK_1 and tachykinin NK_2 receptors, atropine (1 μM), an antagonist of muscarinic acetylcholine receptors, suramin (400 μM), an antagonist of purinocetors, naloxone (100 μM), an antagonist of opioid receptors (100 μM), and tetrodotoxin (1 μM), a blocker of voltage-dependent sodium channels on neurons on EFS-induced contraction, in the post-inflamed hamster colon. EFS was applied using multi-pulses of 20 V each, with a 0.3-ms pulse duration at 20 Hz for 1 s; longitudinal mechanical responses were recorded isometrically. *Circles* Points of electrical stimulations. **b** Summary graphs of the effects of L-732,138 (1 μM), SR48968 (1 μM), atropine (1 μM), suramin (400 μM), naloxone (100 μM) and tetrodotoxin (1 μM) on EFS-evoked contraction in the post-inflamed hamster colon ($n = 10$). Each value represents the mean ± SD. $^{**}p < 0.01$ compared to the control, which is the response before addition of blockers

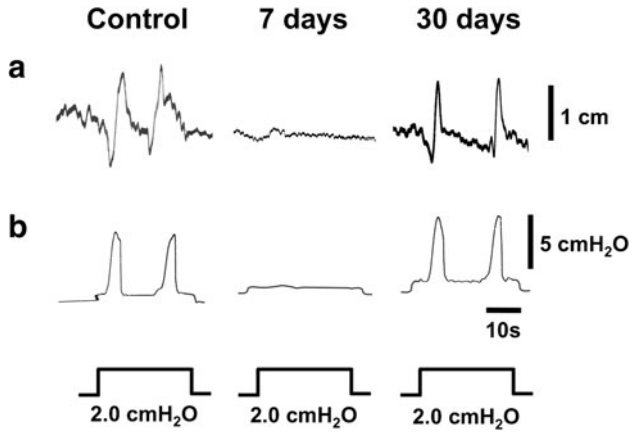

Fig. 8 Effects of inflammation on peristaltic responses to intraluminal distension in intact and inflamed hamster colons. Peristalsis was induced using the Trendelenburg method. Intraluminal pressure stimulation (2 cmH_2O) was given for 30 s. Relaxation and contraction responses in longitudinal muscle, representing the preparatory phase of peristalsis (**a**), and intraluminal pressure changes, representing the empty phase of peristalsis (**b**), were recorded

receptors. Similarly, El-Mahmoudy et al. [33] showed that tachykinins are the main mediators for NANC excitatory neurotransmission and that their action is achieved by both NK_1 and NK_2 receptors in the hamster ileum. Taken together, it can be concluded that activation of the enteric nerves by EFS in the isolated hamster colon can elicit both nitrergic relaxation and cholinergic and tachykininergic contraction.

In the present study, colonic damage induced by TNBS was maximal over the first week. EFS induced no or little mechanical response in the distal colon at 7 days after TNBS treatment (Fig. 5). It has been demonstrated in different experimental models of colitis that the inflammatory process strongly affects several populations of enteric nerves, including cholinergic, adrenergic, nitrergic, VIPergic, tachykinergic and purinergic neurons [7, 13, 14, 24, 34]. Therefore, the loss of mechanical responses to EFS after induction of colitis would be attributable to impairment of the neural function of enteric nerves. This is supported by the results of our previous study showing that contractility of longitudinal smooth muscles in the inflamed colon of the hamster in response to exogenous application of acetylcholine itself remains intact [27]. In accordance with this, we found that both preparatory and empty phases of peristaltic motility were disrupted in the colon at 7 days after treatment with TNBS (Fig. 8). It should be noted, however, that this idea does not necessarily rule out the possibility of damage to smooth muscles. In fact, it has been reported that myogenic contractions induced by high K^+ and carbachol were decreased in rats with TNBS-induced colitis [35].

Damage to the colon observed in the acute inflammatory phase (7 days post-TNBS treatment) was resolved at 30 days after TNBS treatment as judged by macroscopic observation. This is consistent with results of previous studies in which recovery of the inflamed colon was assessed by measurements of activities of inflammation-related enzymes, such as myeloperoxidase, in addition to microscopic observation [9, 10, 36]. Sanovic et al. [10] showed that intestinal inflammation induced rapid axonal proliferation within longitudinal and circular muscle layers, which superimposed to neuronal loss and contributed to the maintenance of innervation density during and after inflammation. Furthermore, the recovery of myenteric plexus organization can be proven by reappearance of

neuronal markers, such as S100 protein or PGP9.5 [9]. In agreement with results of those previous studies, impairment of neurally mediated contractile responses in the inflamed colon was no longer observed in the previously inflamed colon (30 days post-TNBS treatment) in our study. Collectively, it is reasonable to assume that colonic inflammation and its related disorders are resolved by 30 days after TNBS treatment. However, the apparent recovery from inflammation is not necessarily accompanied by complete restoration of neural components. In contrast to low-frequency EFS (2 Hz)-induced responses (see Fig. 6), combined application of tachykininergic and cholinergic antagonists failed to suppress high-frequency EFS (20 Hz)-induced contraction in the colon preparation from the recovered hamster (Fig. 7). This lack of suppression indicates that the non-cholinergic/non-tachykininergic excitatory neural components can be expressed during the course of recovery, although the excitatory cholinergic/tachykininergic neural components are restored at least in part.

The results of the pharmacological experiments in this study revealed that the compensatory neural components, which are manifested in the previously inflamed colon, are purinergic and opioidergic components. Purinergic (P2X3 receptor-mediated) mechanosensory transduction, which might be related with nociception, has been shown to be enhanced in a rat model of colitis [25]. In human IBD patients, there is a significant increment of P2X3 receptor-immunoreactivity in the inflamed colon [26]. Similarly, up-regulation of expression of mu-opioid receptors, together with increased expression of endogenous opioids, such as beta-endorphin and Met-enkephalin, has been demonstrated in the inflamed colon of animal models [20, 23] and IBD patients [21, 22]. On the other hand, using electrophysiological methods, Strong et al. [24] showed that purinergic inhibitory neuromuscular transmission is selectively attenuated in ulcerated regions of the inflamed guinea pig distal colon. These observations collectively suggest that purinergic and opioidergic components in the enteric nervous system change during inflammation of the colon in general. However, the functional roles of changes in purinergic and opioidergic neural components in the regulation of colonic motility have not yet been documented. To our knowledge, our investigation is the first study to reveal that purinergic and opioidergic neural components can compensate for inflammation-induced disruption of motor activity in the previously inflamed colon.

In contrast to the excitatory neural components, the inhibitory neural component was consistently involved in the release of NO during the course of recovery. This indicates that the time course and/or pattern of recovery of neural components from inflammatory damage are different, although there is an indiscriminate loss of myenteric neurons in TNBS-inflamed colons [37]. Expanding the behavior of each neural component following the resolution of colitis would be useful for a logical construction of pharmacological treatments for post-inflammatory disorders in the gut. The hamster might be a suitable colitis model to investigate post-inflammatory changes of enteric neurons, especially excitatory ones, clinically as well as basically.

In summary, we have demonstrated the expression of purinergic and opioidergic excitatory neural components in addition to the reappearance of excitatory cholinergic and tachykininergic and inhibitory nitrergic components in the hamster colon following the resolution of TNBS-induced colitis. This may explain the mechanism of compensation for functional disorders sustained for a long period after recovery of colitis.

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Conflict of interest The authors declare that they have no conflicts of interest.

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