Nicotine-Induced Neurogenic Relaxation in the Mouse Colon: Changes With Dextran Sodium Sulfate–Induced Colitis

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Abstract. Nicotine has been shown to reduce both tone and muscular activity in the human colon by releasing nitric oxide (NO) from nerves. To our knowledge, however, the effect of nicotine on mouse colon has not been elucidated, and the response in tissue from ulcerative colitis (UC) has not been investigated. We examined nicotine-induced responses in colon from control mice and mice with dextran sodium sulfate (DSS)-induced UC. In controls, bath application of nicotine caused a transient relaxation in longitudinal preparations from the transverse and distal colons but not from the rectum. The response was observed in the presence of bethanechol, abolished by treatment with tetrodotoxin and hexamethonium, and mediated partially (>50%) by the NO pathway. In longitudinal preparations of the distal colon from DSS-treated mice, spontaneous contractions decreased markedly, and nicotine caused contraction without relaxation in half of the preparations tested. Nicotine-induced relaxation in the presence of bethanechol was significantly decreased in the DSS-treated distal colon without changing bethanechol-induced contractions. These data suggest that 1) responses to nicotine differ dependent on colon regions, 2) DSS treatment predominantly caused nicotine-sensitive neurogenic changes in distal colon, and 3) DSS treatment may reverse the direction of nicotine-evoked responses in the colon, in mice.

Keywords: mice distal colon, motility disorder, nicotine, nitric oxide, ulcerative colitis

Introduction

Ulcerative colitis (UC) and Crohn’s disease are classified as inflammatory bowel diseases, a general term for chronic inflammation of the gastrointestinal tract. Patients with inflammatory bowel disease suffer from various symptoms including diarrhea and urgent defecation. Although inflammatory bowel disease is associated with defects of barrier permeability and immune function in the gastrointestinal tract, there is often no correlation between the severity of symptoms and the degree of inflammation in tissues (1, 2). Other mechanisms including neuronal disturbances of motor and/or sensory functions in the colon may in part contribute to the symptoms of inflammatory bowel disease. Mucosal inflammation alters nerve and muscle function in models of colitis in vitro (3). Smokers have a lower incidence of some neurodegenerative conditions such as Parkinson’s disease and gastrointestinal disorders such as UC and pouchitis, although some diseases including Crohn’s disease and peptic ulcer disease are worse in smokers (4, 5). Clinical trials in nonsmokers with active UC show that transdermal nicotine reduces symptoms such as diarrhea and urgency without significantly influencing inflammation (5). In terms of its potential for use in the treatment of UC, nicotine-based therapy is of great interest (5, 6).

Numerous studies have shown that nicotine causes smooth muscle to relax and/or contract at various sites in the gastrointestinal tract in various species. For
instance, treatment with nicotine produced a contraction of the longitudinal muscle of the ileum that was mediated by the release of acetylcholine (ACh) in rats (7) and guinea pig (8). In isolated proximal and distal colons from rats (9), proximal colon from dogs (10), and opossum ileocolonic junction (11), treatment with nicotine caused neurogenic relaxation. Relaxation induced by nicotine has also been reported in the human colon (4, 12 – 14), although nicotine caused contractions with the release of ACh in circular preparations from human colon (7). Nicotine-induced contractions have been established in the jejunum and ileum in mice (15 – 17), although Okishio et al. (16) reported that nicotine induced relaxation of circular muscle of the mouse ileum in the presence of atropine. However, it remains unknown whether treatment with nicotine induces contractions in the mouse colon and rectum to our knowledge. Mice are becoming increasingly important subjects for studying functions in the gastrointestinal tract including colon under physiological and pathological conditions. The reasons for this are the availability of mutants, the advance of molecular technology for gene targeting, and the ease of handling for various experiments in vivo. In order to estimate region-dependent changes in the effect of nicotine in the present study, we examined the nicotine-induced contractile response in the transverse colon, distal colon, and rectum.

To investigate the effect of inflammation on intestinal motility, various animal models of experimental colitis have been used (18 – 20). Dextran sodium sulfate (DSS)-induced colitis is one model that shares the clinical and morphological features of human UC. A characteristic feature of this model is that colitis is dominant in the colon, and the DSS-induced inflammation appears in the mucosa/submucosa only (18, 19). In human UC, it is reported that nicotine treatment changed the tone and activity of the colon via the nitric oxide (NO) pathway (4, 12). However, the precise changes in the contractile response induced by nicotine in colitis have not been established in humans or animal models. Therefore, we investigated the change of nicotine-evoked responses in the distal colon from mice having DSS-induced colitis. We show that bath application of nicotine caused a transient relaxation in longitudinal preparations from the colon (transverse and distal colons) in a hexamethonium (C6)- and tetrodotoxin (TTX)-sensitive as well as NO-dependent manner. In longitudinal preparations from the rectum, electrical stimulation (ES), but not treatment with nicotine, caused relaxation via the NO pathway. There was a marked impairment and/or change of nicotine-induced contractile responses in the distal colon from mice with DSS-induced colitis.

Materials and Methods

Animals and induction of experimental colitis

Male ddY mice were purchased from SLC Co. (Shizuoka). Animals, weighing 34 – 41 g, in groups of 5 or 6, were used. They were housed under controlled environmental conditions (temperature of 24 ± 2°C and lights on between 7:00 a.m. and 7:00 p.m.) and fed commercial MF chow (Oriental Yeast Co., Ltd., Tokyo) for at least 1 week before the experiments. Before the experiments, mice were kept individually and fasted for 16 – 18 h with free access to water. They were stunned before being decapitated and bled. Colitis was induced by adding DSS (M.W. 5000; Wako, Osaka) to their drinking water to a final concentration of 2.5% as described previously (21). Animals with diarrhea and bloody excrement were killed from 7 – 10 days following the DSS treatment, and the decrease of body weight was within 15% – 25%, but not over 25%, of that before the treatment. Since reactivity to DSS treatment differed slightly between individual animals, we used those animals satisfying the above criteria. Housing and handling of animals were performed in accordance with the Guide Principles for the Care and Use of Laboratory Animals approved by The Japanese Pharmacological Society, and the experiments were approved by the Graduate School Committee of Chiba University.

Reagents

The following drugs and chemicals were used: ACh chloride, C6 chloride, Nω-nitro-L-arginine methyl ester (L-NAME), and bethanechol (Sigma, St. Louis, MO, USA); nicotine tartrate (Tokyo Kasei, Tokyo); TTX (Wako, Osaka); atropine sulfate and L-arginine (Nacalai, Kyoto); and prostaglandin E2 (PGE2; Cayman, Ann Arbor, MI, USA). PGE2 was dissolved in dimethylsulfoxide, and the other reagents were dissolved in distilled water prior to dilution in Krebs-Henseleit buffer. The vehicles had no effect on the contraction of the colon and ileum in the presence or absence of nicotine or PGE2.

Preparations and measurement of contractile response

The entire length of colon was removed in Krebs-Hanseleit buffer (112.0 mM NaCl, 5.9 mM KCl, 2.0 mM CaCl2, 1.2 mM MgCl2, 2.0 mM NaH2PO4, 25.0 mM NaHCO3, 11.5 mM glucose, pH, 7.4). Whole segments (approximately 1 cm in length) of the transverse colon and distal (descending) colon were taken 2 – 3.5 and 3.5 – 5.5 cm, respectively, from the ileo-caecal junction. The segment, up to 1 cm in length from the anal orifice, was used as rectum. The preparations (including mucosa, circular layer, and neuronal plexus) were usually sus-
suspended in the longitudinal direction under a 1-g load in a 5-ml organ bath containing the buffer. The bath was maintained at 37°C and continuously bubbled with a mixture of 95% O₂ and 5% CO₂. In some experiments, the preparations suspended along the circular axis (the ring preparations) from the distal colon and rectum were used to investigate contractile responses in the whole circular muscle preparations. One end of each segment was attached to an isometric transducer (T-7-8-240; Orientic Co., Tokyo), and recordings were made with a recorder (056; Hitachi, Mito) via a DC strain-amplifier (6M92 or AS2102; NEC San-ei, Tokyo). The other end was mounted on a rigid support or on an anodal electrode placed at the bottom of the bath. The reagents were added to the organ bath. At the start of each experiment, the maximum response to 3 μM ACh was measured in each preparation to evaluate the effects of the reagents tested such as nicotine and PGE₂. For treatment with the inhibitors or receptor antagonists, the preparations were incubated for the indicated period with the reagents, after which the response induced by nicotine was measured in the presence of the respective reagents. The inhibitors or antagonists were used at concentrations reported previously (8, 22). A stimulator (S9; Grass Instrument, Quincy, MA, USA) was used for ES (electrical transmural stimulation, 30-V intensity, 0.1-ms duration, and 10-Hz frequency for 5 s), and the responses were recorded isometrically with a 1-min interval between tests. The conditions for the modified ES used in rectum preparations were 10 V, 0.2-ms duration, 200-ms interval, and 50 trains. Each contractile response was expressed as a percentage of the contraction induced by 3 μM ACh (% of ACh contraction) in many cases, and absolute values of contraction are shown in Table 3. In some cases, the data were normalized as percentages of the corresponding control responses to the indicated reagents. The use of isotonic transducers allowed us to record contraction as changes in smooth muscle elongation under constant tension, thus minimizing possible inflammation-induced and/or individual variations of intrinsic contractile activity of muscles. In some data in Fig. 7 and Table 3, the contractile response was recorded using an isotonic transducer (Type 45347, NEC Sanei).

**Statistical analyses**

Values are presented as means ± S.E.M. for three or more independent experiments. The number in experiments (n) refers to the number of experimental animals used. The statistical significance of differences between two groups was assessed using the two-tailed Student’s t-test. Multiple comparisons against a single control group were made by a one-way analysis of variance followed by Dunnett’s test. P<0.05 or P<0.01 was considered significant.

**Results**

**Nicotine-induced relaxation in longitudinal preparations from mouse colon**

First, we examined the effect of exogenously added nicotine on longitudinal preparations with the myenteric plexus of the transverse colon, the distal colon, and the rectum from mice. The doses of nicotine chosen were close to plasma concentrations in smokers and were based on previous reports (13, 14, 23). Stimulation with nicotine at 1 μM and above induced relaxation of the longitudinal preparations from the distal colon (Fig. 1A), as in colons from other species including humans (4, 9, 10, 13, 14). The nicotine-induced relaxation started within 5 s after treatment, and the character of the response varied depending on the preparation; the responses were transient within 10 s or relatively sustained over 20 s, and maximal relaxation was 5%–
20% in each preparation. Treatment with nicotine induced relaxation in the transverse colon (Fig. 1B) but not in the rectum (Fig. 1C). In rat colon, it is reported that nicotine-induced relaxation was prone to tachyphylaxis (9). In the mouse distal and transverse colons, however, the responses induced by nicotine in repeated trials were almost constant at least in 3 – 4 trials in an experiment within 60 min; the repeated responses induced by 10 μM nicotine were almost the same as in the first trial (5% – 20% relaxation).

Next we investigated the effect of nicotine on the contractile response in the presence of 3 μM PGE<sub>2</sub> (Fig. 2). The sensitivity (affinity and magnitude of contraction) to PGE<sub>2</sub> in the distal colon and in the transverse colon was similar; the ED<sub>50</sub> values were approximately 0.5 μM and the maximal responses were 60 – 70% (of ACh response) in both the colons. The maximal contraction by PGE<sub>2</sub> in the rectum was 40% – 50%, somewhat smaller compared with that in the distal colon. Treatment with 1 μM nicotine caused marked and significant relaxation in the distal colon and in the transverse colon, but not in the rectum (Fig. 2). Stimulation with 3 and 10 μM nicotine also caused relaxation in the distal and transverse colons (peak response, 15% – 30% of ACh response), but the response was small (<5%) even at 10 μM nicotine in the rectum. The relaxation induced by nicotine in repeated trials was resistant to tachyphylaxis in the presence of PGE<sub>2</sub>, like that without PGE<sub>2</sub>. The relaxation induced by nicotine was almost completely inhibited in the 300 μM C6– and the 1 μM TTX–treated preparations from the distal colon and from the transverse colon (Fig. 3 and Table 1). The relaxation induced by nicotine was not inhibited by 1 μM atropine, although the contraction induced by 3 μM ACh

Table 1. Effects of C6 and TTX on nicotine-induced relaxation in the mouse distal colon

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Nicotine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 μM</td>
</tr>
<tr>
<td>Exp. I.</td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>18.3 ± 3.3 (5)</td>
</tr>
<tr>
<td>C6</td>
<td>0&lt;sup&gt;a&lt;/sup&gt; (5)</td>
</tr>
<tr>
<td>Exp. II.</td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>20.9 ± 2.4 (6)</td>
</tr>
<tr>
<td>TTX</td>
<td>1.0 ± 1.0&lt;sup&gt;a&lt;/sup&gt; (6)</td>
</tr>
</tbody>
</table>

The longitudinal preparations were incubated with vehicle, 300 μM C6, or 1 μM TTX for 10 min and then stimulated with the indicated concentrations of nicotine in the presence of 3 μM PGE<sub>2</sub>. The contractions induced by PGE<sub>2</sub> in the C6- and TTX-treated preparations were almost the same as those in the vehicle-treated preparations. (n), number of preparations from different mice. *P<0.05, significantly different from the vehicle-treated value.
was completely inhibited by the atropine treatment (data not shown). Although the dose-dependency of nicotine-induced relaxation was not clear from the peak response, the areas for the response to 10 μM nicotine were about 1.3-fold in the distal colon (Fig. 4D) and 1.8-fold in the transverse colon (data not shown), compared with that to 1 μM nicotine in the colons. Stimulation with 0.1 μM nicotine had no effect and 0.3 μM nicotine had only a limited effect on the preparations from each region (data not shown). A role for cyclooxygenases is proposed in the modulation of neuromuscular functions in the mouse and human distal colon (24). However, treatment of the distal colon with 10 and 20 μM indomethacin for 30 min did not change the relaxation induced by nicotine; the responses induced by 1 and 3 μM nicotine in the indomethacin-treated preparations were 21% (peak relaxation) and 28%, respectively, which were almost the same as those in the control in a typical experiment (n = 2).

Role of NO pathway in nicotine-induced relaxation in colons

There is a consensus that NO makes a major contribution to the inhibition of contractile responses and/or relaxation of smooth muscles in the gastrointestinal tract including the colon. Treatment with 1 mM L-NAME for 10 min decreased the relaxation induced by nicotine in the distal colon (Fig. 4). Next, L-NAME–treated preparations were washed with L-NAME–free buffer and then further incubated with 3 mM L-Arg for 10 min. Treatment with L-Arg reversed the nicotine-induced relaxation in the distal colon; the treatment almost completely reversed the nicotine-induced response (peak and area values, shown as % of the control value). Similar results were obtained in the transverse colon (data not shown).

Distal colon

![Figure 4](image-url)

**Fig. 4.** Effects of L-NAME and L-Arg on nicotine-induced relaxation in longitudinal preparations of the distal colon. The responses induced by nicotine in the presence of 3 μM PGE2 were examined in the distal colon. Then, the same preparations were treated with 1 mM L-NAME for 10 min and the responses induced by PGE2 and nicotine were examined. The washed preparations were treated with 3 mM L-Arg for 10 min, and the responses were examined. Since the contractile responses were examined for a longer period in these experiments, 1 mM L-NAME was required and 3 mM L-Arg was used in order to antagonize the L-NAME effect. Treatment with neither L-NAME nor L-Arg modified the PGE2-induced response. The recordings in panel A are a typical example of 3–7 independent experiments. Quantitative data are shown in panels B–E. The peak responses of nicotine-induced relaxation (% of PGE2 response) are shown in panel B. The total responses (area = peak × time, % of control) are shown in panel D. In panels C and E, each response was expressed as a percentage of the respective response induced by 1, 3, and 10 μM nicotine. aP < 0.05, significantly different from the value without nicotine; bP < 0.05, significantly different from the control value without L-NAME; cP < 0.05, significantly different from the value with L-NAME. (n), number of preparations from different animals.
Involvement of NO pathway in ES-induced relaxation in distal colon and rectum

Next, we investigated whether the NO pathway exists or not in mouse rectum. The ES caused first a relaxation followed by a contraction of the longitudinal preparations from the distal colon (Fig. 5A), and treatment with 100 μM L-NAME almost completely abolished the ES-induced relaxation and enhanced the contraction. In mouse rectum, stimulation with ES caused marked relaxation and subsequent contraction, and treatment with 200 μM L-NAME abolished the relaxation and enhanced the contraction induced by ES (Fig. 5B). Treatment of the L-NAME–treated preparations with 2 mM L-Arg abolished the L-NAME–induced responses in distal colon and rectum. Quantitative data concerning the existence of the NO pathway in the mouse rectum are shown in Table 2.

Nicotine-induced relaxation of circular preparations from distal colon and rectum

Under our conditions, the circular preparations from both the distal colon and rectum showed marked spontaneous contraction (Fig. 6). Treatment with 10 μM

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Table 2. Effects of L-NAME and L-Arg on ES-induced responses in longitudinal preparations from the mouse distal colon and rectum

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>L-NAME</th>
<th>L-Arg after L-NAME</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distal colon (2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>contraction (peak)</td>
<td>100%</td>
<td>200, 166</td>
<td>100, 85</td>
</tr>
<tr>
<td>relaxation (peak)</td>
<td>100%</td>
<td>30, 35</td>
<td>80, 105</td>
</tr>
<tr>
<td>Rectum (3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>contraction (peak)</td>
<td>100%</td>
<td>157 ± 17 a</td>
<td>122 ± 15 b</td>
</tr>
<tr>
<td>(area)</td>
<td>100%</td>
<td>184 ± 22 a</td>
<td>99.0 ± 19.1 b</td>
</tr>
<tr>
<td>relaxation (peak)</td>
<td>100%</td>
<td>14.7 ± 4.4 a</td>
<td>113 ± 24 b</td>
</tr>
<tr>
<td>(area)</td>
<td>100%</td>
<td>4.5 ± 4.5 a</td>
<td>142 ± 35 b</td>
</tr>
</tbody>
</table>

The longitudinal preparations of distal colon and rectum were stimulated with ES as described in Materials and Methods. Then, the same preparations were treated for 10 min with 100 μM (distal colon) and 200 μM L-NAME (rectum), and the ES-induced responses were examined. The washed preparations were treated with 2 mM L-Arg for 10 min, and the ES-induced responses were examined. The recordings shown in Fig. 5 were one typical example of 2 – 3 independent experiments. Quantitative data were expressed as a percentage of control values. aP<0.05, significantly different from the control value without L-NAME. bP<0.05, significantly different from the value for the L-NAME–treated preparations.

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Fig. 5. ES-induced relaxation and contraction in longitudinal preparations of the distal colon (A) and rectum (B). The contractile responses were measured with ES. The conditions for ES were described in Materials and Methods. Since the conditions for ES were different in the distal colon and the rectum, the relaxation in the distal colon was not marked. The same preparations were treated with the indicated concentrations of L-NAME for 10 min, and the responses induced by ES were examined. Since ES-induced responses were examined for a short period, the effects of L-NAME were examined with low concentrations (100 or 200 μM). Quantitative data are shown in Table 2.

Fig. 6. Nicotine-induced relaxation in circular preparations of the mouse distal colon (A) and rectum (B). The circular preparations were stimulated with 10 μM nicotine. On the right, the preparations were pretreated for 10 min with 300 μM C6 and then stimulated with 10 μM nicotine. The recordings are a typical example of 2 independent experiments.
nicotine decreased spontaneous contraction and caused a slight relaxation (10% – 20% of ACh response) in the distal colon (Fig. 6A). Nicotine treatment caused a marked relaxation, >30% of ACh response, in circular preparations from the rectum (Fig. 6B). The responses to nicotine in both regions were almost completely inhibited in the 300 μM C6–treated preparations. Since the spontaneous contraction was large and the response by nicotine was not marked in the circular preparations, the role of the NO pathway in nicotine-induced relaxation could not be determined.

Changes of nicotine-evoked response in the longitudinal preparations of distal colon from DSS-treated mice

The DSS-induced colitis in mice was used as an experimental model to study the changes in contraction of the longitudinal preparations during inflammation. Mice treated with 2.5% DSS developed bloody diarrhea 5 – 7 days later. Although the mean body weight for DSS-treated mice at 2 – 4 days was increased like that for control mice, it significantly decreased 7 – 8 days after the treatment (Table 3). Mean colon length was decreased 8% – 15% in the DSS-treated mice (data not shown), as described previously (20, 25). The distal colon from control mice exhibited spontaneous phasic contractions (Figs. 1 and 7A), but the response in the colon from DSS-treated mice markedly decreased (Fig. 7 and Table 3). Interestingly, the relaxation induced by 10 μM nicotine decreased in all the preparations from the DSS-treated distal colon tested, and nicotine treatment caused a contraction following relaxation in some preparations from DSS-treated mice (Fig. 7A, Table 3).

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>DSS-treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Changes from 0 day</td>
<td>+6.9 ± 0.4</td>
<td>–4.8 ± 0.8b</td>
</tr>
<tr>
<td>Changes from the peak</td>
<td></td>
<td>–7.6 ± 0.9b</td>
</tr>
<tr>
<td>A: Responses in isometric transducer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spontaneous (mg)</td>
<td>57.8 ± 10.1</td>
<td>19.0 ± 8.3b</td>
</tr>
<tr>
<td>Nicotine-induced relaxation (mg)</td>
<td>103 ± 35</td>
<td>45.5 ± 18.4</td>
</tr>
<tr>
<td>Nicotine-induced contraction</td>
<td>0/4</td>
<td>4/7</td>
</tr>
<tr>
<td>ACh response (mg)</td>
<td>1636 ± 222</td>
<td>1295 ± 181</td>
</tr>
<tr>
<td>pED50</td>
<td>–5.9 ± 0.2</td>
<td>–5.7 ± 0.2</td>
</tr>
<tr>
<td>Bethanechol response (mg)</td>
<td>1793 ± 303</td>
<td>1507 ± 68</td>
</tr>
<tr>
<td>Nicotine-induced relaxation in the presence of bethanechol relaxation (peak, % of bethanechol)</td>
<td>43.5 ± 3.2</td>
<td>26.7 ± 2.9b</td>
</tr>
<tr>
<td>T1/2 (min)</td>
<td>0.8 ± 0.2</td>
<td>2.5 ± 0.6b</td>
</tr>
<tr>
<td>B: Response in isotonic transducer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nicotine response (% of ACh)</td>
<td>5.9 ± 1.6</td>
<td>0.9 ± 0.5a</td>
</tr>
<tr>
<td>Nicotine-induced contraction</td>
<td>0/6</td>
<td>4/4</td>
</tr>
<tr>
<td>Nicotine-induced relaxation in the presence of bethanechol relaxation (peak, % of bethanechol)</td>
<td>31.3 ± 6.3</td>
<td>15.3 ± 8.1</td>
</tr>
<tr>
<td>T1/2 (min)</td>
<td>1.1 ± 0.2</td>
<td>3.9 ± 1.1a</td>
</tr>
</tbody>
</table>

The longitudinal preparations of distal colon from control and DSS-treated mice were stimulated with the indicated reagents. The number of animals is given in parentheses. In experiment A, the contractile activity was recorded isometrically, and an initial resting tension of 1 g was applied to the preparations. The spontaneous activity and the 10 μM nicotine-induced response were measured, and the number of responders showing contraction is shown. The maximal contraction induced by ACh and bethanechol and the pED50 value of ACh are also shown. Nicotine (3 μM)-induced relaxation was examined in the presence of 30 μM bethanechol, and the peak response was expressed as a percentage of the bethanechol-induced contraction. The half-time (T1/2) of the recovery from the maximal relaxation induced by 3 μM nicotine was calculated. In some DSS-treated preparations, nicotine-induced relaxation was sustained, and thus the T1/2 values were calculated as 5 min. In experiment B, the contractile activity was recorded isotonically as described in the Materials and Methods. Nicotine-induced relaxation was expressed as a percentage of the ACh-evoked response. *P<0.05, bP<0.01, significantly different from the control.
Change of Nicotine Response in Colitis

Table 3), although nicotine treatment caused a relaxation, not contraction, in the distal colon from control mice. The contraction of the preparations from DSS-treated mice was almost completely inhibited by 100 nM atropine and 1 μM TTX (data not shown). The maximal contraction induced by ACh and bethanechol in the DSS-treated colon decreased slightly, but not significantly, compared with that in the control. The pED$_{50}$ values of ACh (Table 3) and bethanechol (data not shown) in the distal colon from DSS-treated mice were almost the same as those for control mice. Treatment with a Ca$^{2+}$ ionophore (5 μM A23187) caused a marked contraction to a similar degree in both the control and DSS-treated distal colon (data not shown).

In order to show the changes in the response to nicotine in the DSS-treated distal colon more clearly, we investigated the response in the presence of PGE$_2$ and bethanechol. In half of the DSS-treated distal colon, the contraction induced by 3 μM PGE$_2$ rapidly returned to the basal level compared with the control, although the initial peak contraction induced by PGE$_2$ in the DSS-treated colon was almost the same as that in the control. The contraction induced by PGE$_2$ in the DSS-treated distal colon was susceptible to desensitization; the peak contraction induced by a second exposure to PGE$_2$ was half of the initial exposure (manuscript in preparation).

Next, we examined the response to nicotine in the presence of 30 μM bethanechol (Fig. 7B). The peak response of 3 μM nicotine–induced relaxation in the DSS-treated distal colon was significantly low compared with that in the control. Although the nicotine-induced relaxation was transient in the control colon, the response in the DSS-treated colon was sustained; the half-time (T$^{1/2}$) of the recovery from the maximal relaxation induced by 3 μM nicotine was less than 1 min in the control colon, but was significantly longer in the DSS-treated colon (Table 3). Similar changes in the DSS-treated colon were observed in the presence of 5 μM bethanechol or 10 μM ACh instead of bethanechol (data not shown). Impairment of this response and an opposite response (contraction) to nicotine in DSS-treated colon were observed when the contractile response was recorded using an isotonic transducer (Fig. 7, C and D, and Table 3). As described above, the sensitivity to nicotine of the longitudinal preparations was low and/or not detected in the rectum. Thus, the distal colon was further separated into two parts (that close to the transverse colon and that close to the rectum), and the responses in the two parts were measured. The responses to nicotine and the changes induced by DSS treatment were almost same in the two parts of distal colon. For example, spontaneous contractions (mg) of distal colon close to the rectum measured by an isometric transducer were 70.7 ± 5.6 (n = 10) and 15.5 ± 5.5 (n = 10) in the control and the DSS-treated mice, respectively; and the half of the colon close to rectum from the DSS-treated mice showed nicotine-induced contraction, not relaxation. Similar data were
obtained in distal colon close to the transverse colon from control and DSS-treated mice (data not shown). The spontaneous contraction of the longitudinal preparations of transverse colon was decreased in the DSS-treated mice compared with the control, but the responses to nicotine in the transverse colon were not changed by DSS treatment.

Discussion

Nicotine-induced relaxation of mouse colon but not rectum

As described in the Introduction, treatment with nicotine causes neurogenic relaxation in the colon in various species including rats and humans. However, the response in the mouse colon and rectum has not been established to our knowledge. In the present study, we showed that nicotine at 1 – 10 μM showed that nicotine at 1 – 10 μM significantly caused a rapid relaxation in longitudinal preparations from mouse transverse and distal colons (Figs. 1 and 2). Our data concerning nicotine-induced relaxation of the longitudinal preparations of colon from mice are consistent with the results from rats (9) and dogs (10). In the mouse distal colon, nicotine caused a relaxation both in the longitudinal (Fig. 1) and in the circular preparations (Fig. 6).

Treatments with nicotine had no effect on the contraction and relaxation of longitudinal preparations from the mouse rectum (Fig. 1C), but caused relaxation of the circular preparations from the rectum (Fig. 6B). In the human sigmoid colon, treatment with 10 μM nicotine reduced the tone and amplitude of spontaneous contractions of the circular muscles (13, 14), but had no effect on the contraction of longitudinal muscles (14). As discussed below, a functional NO pathway existed in the mouse rectum. The expression of cannabinoid CB1 receptors, which inhibit release of ACh from enteric neurons, was quite low in the mouse rectum compared with that in the colon (26). In the human sigmoid colon, an agonist of the CB1 receptor inhibited ES-induced contraction in circular, but not longitudinal, muscle preparations, although CB1 receptor–immunoreactive fibers were observed in both preparations (27). Thus, the existence and/or coupling of nicotinic ACh receptors, which regulate the contractile response in longitudinal muscles, may be low in the mouse rectum, although the response to nicotine was intact in the circular preparations of the rectum. These findings suggest that the nicotine-induced contractile response in intestinal tissues is dependent on species, tissues, and muscle types.

Role of the NO pathway in nicotine-induced relaxation

Nicotine-induced relaxation of the longitudinal preparations from mouse colon was inhibited by treatment with TTX and C6, but not with atropine (Fig. 3 and Table 1). Thus, the response was mediated by nicotinic ACh receptors on neurons, not by a direct effect on smooth muscles. The data concerning L-NAME and L-Arg showed a role of the NO pathway in nicotine-induced relaxation (Fig. 4). In the human sigmoid colon, nicotine reduced both spontaneous tone and peak tension and relaxation of longitudinal preparations from the rectum in control mice (28). The weaker effect of nicotine in the mouse rectum was not due to an absence of the NO pathway in the preparation since the ES was almost the same as those in the rectum in control mice (28). The weaker effect of nicotine in the mouse rectum was not due to an absence of the NO pathway in the preparation since the ES caused relaxation in the rectum in a L-NAME–sensitive manner (Table 2). Thus, the weak effect of nicotine on the longitudinal preparations from the rectum is not derived from a loss of the NO pathway.

Changes of nicotine-induced response in the DSS-treated mice

In the longitudinal preparations of the distal colon from DSS-treated mice, spontaneous contraction and nicotine-induced relaxation were significantly decreased compared with those in the control (Fig. 7). The decrease in nicotine-induced relaxation in the distal colon from DSS-treated mice was detected both in isometric and in isotonic contractions. It is reported that treatment of the human distal colon with 10μM nicotine reduced both spontaneous tone and peak tension after ES in the control, with less of an effect in the patients with active UC (13). Under our experimental conditions, ACh- and bethanechol-induced contractions, probably via direct activation of muscarinic ACh receptors on the muscles, of mouse distal colon were slightly, but not significantly, impaired by DSS treatment (Table 3). It was reported that DSS treatment suppressed the contraction induced by activation of muscarinic ACh receptors in circular muscle preparations of the distal colon in rats (29) and mice (20). By contrast, other reports found that the contraction induced by muscarinic ACh-receptor activation in circular (19) and longitudinal (30) muscle preparations of rat distal colon were not modified by DSS treatment. In the present study, the Ca²⁺ ionophore–induced contraction in
the longitudinal preparations of the distal colon from DSS-treated mice was almost the same as that in the control. Sato et al. (20) also reported that KCl- and substance P–induced contractions of circular muscles from the mouse distal colon were not changed by DSS treatment. DSS treatment impaired neurogenic and NO-dependent relaxation by ES (19) and by stretch (31) in the rat distal colon. Thus, the DSS treatment used in our study appears to impair neurogenic responses such as spontaneous and nicotine responses without markedly changing muscular responses. The expression of molecules such as inflammatory cytokines and NO synthase induced by DSS treatment was dependent on the progress of colitis which was modulated by experimental conditions such as species including strains of mice, DSS dose and type, and treatment period (20, 25, 30, 32). The inflammation, when severe, appeared to impair not only neuronal but also muscular functions.

A report described contractions induced by nicotine in circular muscle preparations from the human (probably sigmoid or distal) colon via the release of ACh (7). Although it has been established that treatment with nicotine causes contractions in the ileum in various species including mice (7, 8, 15), a recent report showed that nicotine induced relaxation of the mouse ileum in the presence of atropine (16). Interestingly, we found that DSS treatment caused a change from relaxation to contraction of nicotine-induced contractile responses in longitudinal preparations of the distal colon in half of the treated mice. Since the nicotine-induced contraction was inhibited by atropine and TTX, a function of the neurons having nicotinic ACh receptors and releasing ACh appears to exist in the DSS-treated mice. Thus, there may be two types of (inter)neurons having nicotinic ACh receptors in the distal colon, one for relaxation via an NO-dependent pathway and dominant under normal conditions, and another for contraction via the release of ACh and obvious under inflammatory conditions. Although the nicotine-induced relaxation of distal colon was transient and returned to the basal level at 2–3 min after stimulation in the control mice, the response in the DSS-treated mice continued for a longer period and/or normalized slowly (Table 3). The reasons for this are not clear at present. DSS-induced inflammation of the mouse distal colon resulted in selective changes in the Ca²⁺–channel activity of smooth muscle cells (33). Several reports suggest the dysfunction of muscular events in colons from DSS-treated mice in addition to that of the neurons (34, 35). Thus, the change may be derived from dysfunction including an inhibition of Ca²⁺ quenching in muscles.

Conclusion and future problems

In the present study, we showed that activation of nicotine-sensitive neurons caused relaxation in longitudinal (and circular) preparations at least partially via the NO pathway in the mouse distal colon and that the response to nicotine was impaired and/or reversed from relaxation to contraction in the UC model. Our findings may account for the neurogenic colonic motor dysfunction in UC. Many neurotransmitters including substance P and vasoactive intestinal peptide are proposed to be involved in the contractile response in the mouse distal colon (36–38). Nicotine-induced relaxation seemed to be due to the release of vasoactive intestinal peptide-related peptides in the guinea-pig caecum (39) and cat esophageal sphincter (40). In preliminary experiments, nicotine-induced relaxation appeared to be partially (approximately 30%) inhibited in the distal colon treated with 1 μM phenolamine. The specific mediators, including catecholamines, from nicotine-sensitive neurons should be determined. The relationship of nicotine-induced relaxation of longitudinal preparations from colon to a potential beneficial effect of nicotine in UC remains to be solved.

References

9. Qian Y, Jones RL. Inhibition of rat colon contractility by


