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Regionally Differential Effects of Sennoside A on Spontaneous Contractions of Colon in Mice

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Abstract: Sennosides, the most popular irritant laxatives, cause purgative actions in the intestine through biotransformation to rhein anthrone; however, the underlying mechanisms remain unclear. The purpose of this study was to define colonic motor actions of sennoside A with special reference to purgative action. Mice received a single oral dose of 30 mg/kg sennoside A, and the colon was removed about 6 hr later. Contractions of longitudinal and circular muscles were recorded using an isometric force transducer and a pressure transducer, respectively. In longitudinal muscle preparations, spontaneous contractions were augmented in distal colon compared to control. In circular muscle preparations, contractions were reduced in the proximal colon, but increased in the distal colon. Particularly in the proximal colon, the frequency of high-amplitude contraction was reduced. In the control group, non-adrenergic, non-cholinergic treatment decreased the amplitude of contractions in the proximal colon, but not in the distal colon. In the sennoside A group, non-adrenergic, non-cholinergic treatment only slightly depressed the amplitude of contractions in the proximal and distal colon. To confirm a causal relationship between luminal prostaglandin level and purgative action of sennoside A, the mice were treated with indomethacin. Significant changes induced by sennoside A were attenuated by indomethacin treatment. The present study indicates that spontaneous motility is inhibited by sennoside A in the proximal colon, but accelerated in the distal colon, and that effects are associated with luminal prostanoid level and only partially with cholinergic nerve mediation.

Chronic constipation is one of the most common gastrointestinal complaints, and laxatives are sold over-the-counter in many countries. Various kinds of laxatives are available, roughly divided into five groups: bulk or hydrophilic laxatives; surfactant or softening or wetting agents; osmotic laxatives; peristaltic stimulant, sometimes referred to as irritant laxatives and others [1]. Among these, irritant laxatives such as bisacodyl, sodium picosulfate and sennosides have been used in many patients suffering from constipation. However, why irritant laxatives cause purgative actions particularly in association with effects on intestinal motility remains unclear [2].

Sennosides, the most popular irritant laxatives, cause purgative action through biotransformation to rhein anthrone in the intestine [3–6]. Some researchers have reported that the purgative action would result from inhibition of intestinal water and electrolyte absorption [7–9] and by acceleration of large intestinal transit [4,9,10]. As for large intestinal motility, however, others have reported that sennosides inhibit colonic motility, despite developing intermittent spiking activity in dogs [11,12] and rats [13]. The purgative action of rhein anthrone, an active metabolite of sennosides, was inhibited by pretreatment with indomethacin in mice, suggesting an involvement of prostaglandins in the purgative action of sennosides [11,14–16]. In addition, sennosides and their metabolites may act topically in the large intestine, because application of rhein anthrone to the large intestine causes purgative action [14].

These findings for irritant laxatives led us to speculate whether accumulation of watery contents by inhibition of water absorption and depression of mixing movement in the large intestine might induce intermittent spiking activity. Thus, the purpose of this study was to define the mechanisms underlying the purgative actions of sennoside A with special reference to spontaneous colonic motility in mice.

Materials and Methods

Animals. This study examined male ddY strain mice 5–7 weeks old (weight 30–40 g). The mice were maintained under standard environmental conditions (room temperature 24 ± 1°C; relative air humidity 50 ± 2%; a 12-hr light:dark cycle). The animals were treated according to the guidelines for the Care and Use of Animals approved by the Physiological Society of Japan.

Experiments. The mice received a single oral dose of 30 mg/kg sennoside A. At least 5 hr later, symptoms of diarrhoea were checked by examining excretion of wet and/or unformed faeces. When diarrhoea was evident, mice were stunned before being decapitated and bled. The colon was removed from the abdominal cavity and immediately placed in Krebs solution. To evaluate regional differences in spontaneous mechanical activity, we divided the colon into four parts: proximal; mid-proximal; mid-distal and distal colon. The mesenterium was carefully removed from these preparations. Strips (length 7–20 mm) were placed in a 25-ml bath containing Krebs solution at 37°C and bubbled with a mixture of 95% oxygen and 5% carbon dioxide. The segment was cannulated and tied at both ends as

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previously reported [17,18]. Contractions in both directions of the longitudinal and circular muscles were simultaneously recorded using a TB-612T isometric force transducer (Nihon Kohden, Tokyo, Japan) under 0.3 g load and with a BT-AUM-2007 Biotrans2TM pressure transducer (Toray, Tokyo, Japan), respectively. Each transducer was connected to a Mac Lab/8 data acquisition system (software Chart 4.3; AD Instruments, Nagoya, Japan) for continuous recording. Strips were allowed to equilibrate for ≥ 30 min. before experiments started. Spontaneous phasic contractions were recorded with amplitude of contractions assessed as mean peak during a period of 15 min. and contractile events shown as the number of contractions per 15 min. In some series of experiment, the motility index was calculated as the product of amplitude and contractile events. Non-adrenergic, non-cholinergic (NANC) conditions were obtained by adding 1 μM atropine sulfate, 1 μM phentolamine and 1 μM propranolol to the Krebs solution. Effects of each pharmacological agent on amplitude and contractile events were examined before and after drug addition in individual muscle strips. To assess neuronal control level after NANC treatment, spontaneous mechanical activity of muscle strips was further examined in the presence of the nitric oxide synthase inhibitor N-ω-nitro-L-arginine (0.1 mM) and the neural blocker tetrodotoxin (0.1 μM) at each of the four parts of the colon.

To assess the effects of indomethacin on the purgative action of sennoside A via prostaglandin mediation [14,15], the mice were administered indomethacin (5 mg/kg) 2 hr after oral administration of sennoside A (30 mg/kg), then they were killed 4 hr after indomethacin application and the colon was removed. In another series of experiments, mice were given indomethacin (5 mg/kg) and killed 1 hr later. As described above, colonic motility was recorded after ≥ 30 min. equilibration.

Solutions and drugs. Krebs solution comprised: glucose, 11.50 mM; NaCl, 118.00 mM; $NaHCO_3$, 25.00 mM; KCl, 4.7 mM; $NaH_2PO4_2H_2O$, 1.20 mM; $CaCl_2$ $2H_2O$, 2.50 mM and $MgCl_2$ $6H_2O$, 1.20 mM. The following drugs were used: sennoside A (Wako Pure Chemical Industries, Osaka, Japan); N-ω-nitro-L-arginine (Sigma-Aldrich Japan, Tokyo); tetrodotoxin (Wako Pure Chemical Industries); atropine sulfate salt (Sigma-Aldrich Japan); phentolamine mesylate (BBI, Ontario, Canada); DL-propranolol hydrochloride (Nakaraitesuku, Kyoto, Japan) and indomethacin (Sigma-Aldrich Japan). Stock solutions were prepared by dissolving drugs in distilled water, except tetrodotoxin and sennoside A. Tetrodotoxin was diluted in acetate buffer, and sennoside A was dissolved in 0.5% sodium bicarbonate solution. Indomethacin was suspended in 0.5% carboxymethyl cellulose solution.

Data analysis and statistics. Data are expressed as mean ± S.E.M., with the value of n (representing the number of preparations) taken from different animals. In some series of experiments, control data were accumulated from all groups that had the experimental design common to the control. Differences in spontaneous motility between groups were tested using Student's, Aspin-Welch or paired t-tests, and values of $P < 0.05$ were considered statistically significant.

Results

Effect of sennoside A on spontaneous contractions at proximal, mid-proximal and distal colon.

In the sennoside A group, all mice displayed diarrhoea 6 hr after application. Recordings of spontaneous contractions were performed simultaneously using longitudinal and circular muscle preparations at the proximal, mid-proximal and distal colon (fig. 1). Contractions of the mid-distal and distal colon were so similar in pattern (data not shown) that they were analysed as the same regional group. Contractile events were

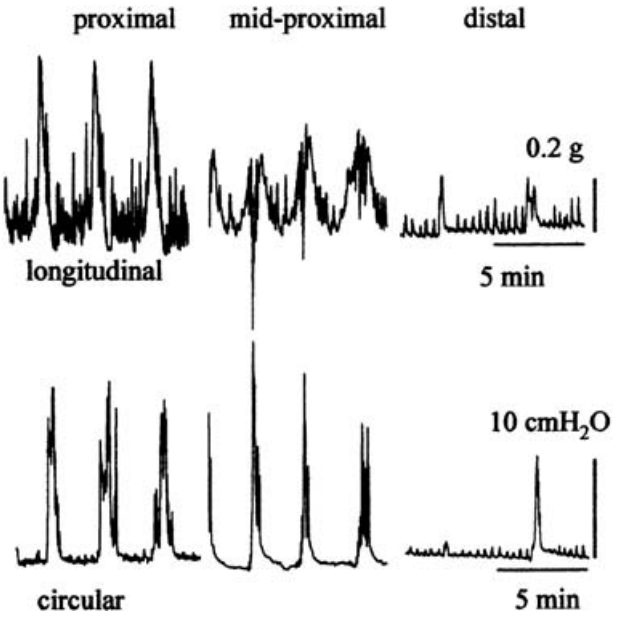

Fig. 1. Simultaneous recordings showing spontaneous contractions of isolated proximal (left), mid-proximal (middle), and distal (right) colon. Longitudinal muscle (upper); circular muscle (lower).

shown as the number of spontaneous contractions > 0.1 g tension in the longitudinal muscle and >1 cm H_2O pressure in the circular muscle. Contractile events of longitudinal and circular muscles at the proximal colon were definitely greater than those shown at the mid-proximal and distal colon in the control group (figs 2 and 3). In contrast, no significant difference in amplitude of contractions was seen among the three regions of colon.

In the longitudinal and circular muscle preparations from mice with diarrhoea induced by sennoside A, the amplitude of contractions was significantly greater in the distal colon than in the control group (figs 2 and 3). In the circular muscle preparations, however, the amplitude of contractions was significantly lower in the proximal colon than in the control group (fig. 3). These changes were not observed when colonic preparations were made from mice recovering from diarrhoea 16 hr after application of sennoside A (data not shown). As for the contractile events of both muscle preparations, no changes in event number were seen compared to the corresponding control group (figs 2 and 3). Although the motor activity was expressed as motility index, the results were intermediate between those shown in events and amplitude (fig. 3). For this reason, the motility index was not shown as a motility parameter in other series of experiments.

Contractions of circular muscle > 10 cm H_2O pressure, designated as high amplitude contractions, were assessed at three colonic regions (fig. 4). In the control group, the contractile events were clearly greater in the proximal colon than in the mid-proximal and distal colon. In the sennoside A group, the contractile events were significantly decreased only at the proximal colon (fig. 4). Changes seen in the proximal colon were attenuated in mice recovering from diarrhoea 16 hr after application of sennoside A (data not shown).

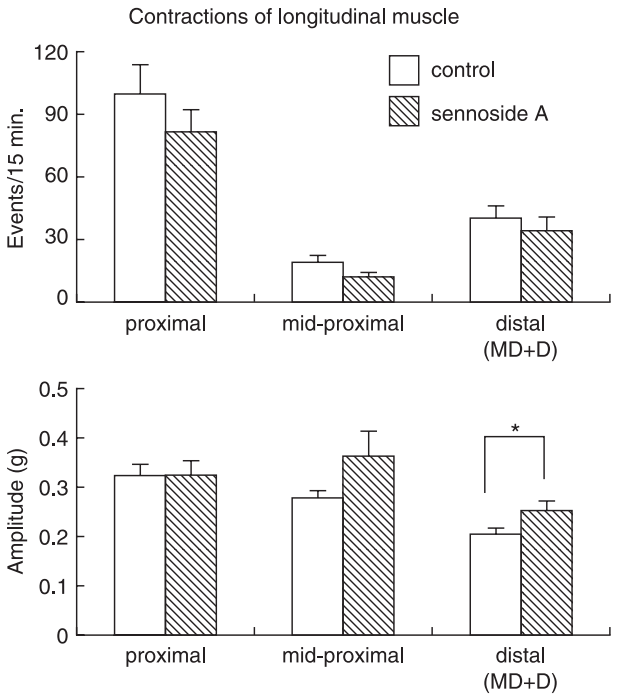

Fig. 2. Effect of orally administered sennoside A on spontaneous contractions of longitudinal muscle in the isolated colon. Spontaneous contractions were augmented in the distal colon in the sennoside A group. Values are given as mean ± S.E.M. (control, n = 21–27; sennoside A, n = 23–30). *P < 0.05, significantly different from control group by the Student's t-test.

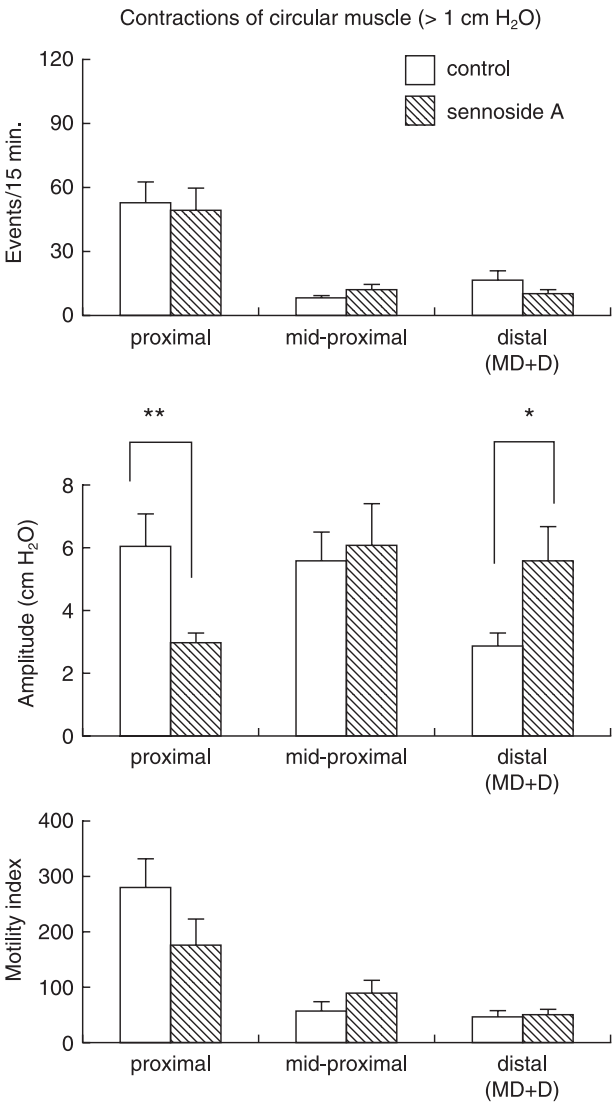

Fig. 3. Effect of orally administered sennoside A on spontaneous contractions of circular muscle in the isolated colon. Spontaneous contractions were reduced in the proximal colon, but increased in the distal colon in the sennoside A group. Values are given as mean ± S.E.M. (control, n = 21–26; sennoside A, n = 22–26). *P < 0.05 and **P < 0.01, significantly different from control group by the Aspin-Welch t-test.

Effect of NANC, N-ω-nitro-L-arginine and tetrodotoxin on sennoside A-induced changes in spontaneous contractions at proximal and distal colon.

In longitudinal muscle preparations of the control group, NANC treatment reduced the amplitude of contractions in the proximal colon, but not in the distal colon (fig. 5). The decreased amplitude was not further modified by treatment with N-ω-nitro-L-arginine or tetrodotoxin. Similar results were observed in circular muscle preparations from the control group. In the sennoside A group, NANC treatment did not significantly depress the amplitude of contractions for longitudinal muscle in the proximal colon, although treatment with N-ω-nitro-L-arginine and tetrodotoxin caused an additional slight decrease (fig. 5). In circular muscle preparations, NANC treatment only slightly depressed the amplitude of contractions in the proximal and distal colon (fig. 5). Subsequent treatments with N-ω-nitro-L-arginine and tetrodotoxin did not further change the contractions in these regions of the colon.

Effect of indomethacin on sennoside A-induced changes in spontaneous contractions at proximal and distal colon.

In this experiment, the effect of indomethacin on spontaneous colonic contractions was assessed regarding significant changes induced by treatment with sennoside A. In the nine mice with sennoside A and indomethacin, four had diarrhoea, but the other five mice did not develop diarrhoea at 6 hr after sennoside A application. In the longitudinal muscle preparations, the amplitude of contractions in the proximal and distal colon in the group with sennoside A and indomethacin did not differ from that in the group with indomethacin (fig. 6). Similar results were observed in circular muscle preparations. The significant changes induced by sennoside A (figs 2 and 3) were thus attenuated by treatment with indomethacin.

Discussion

The present study has shown that sennosides inhibit spontaneous intestinal motility in the proximal colon, but accelerate spontaneous intestinal motility in the distal colon and that

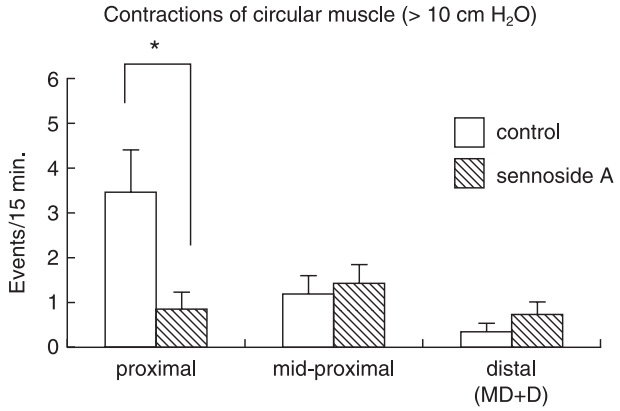

Fig. 4. Effect of orally administered sennoside A on frequency of high-amplitude contractions of circular muscle in the isolated colon. Event number (frequency) of high-amplitude contractions was clearly decreased in the sennoside A group. Values are given as mean ± S.E.M. (control, n = 21–26; sennoside A, n = 22–26). *P < 0.05, significantly different from control group by the Aspin-Welch t-test.

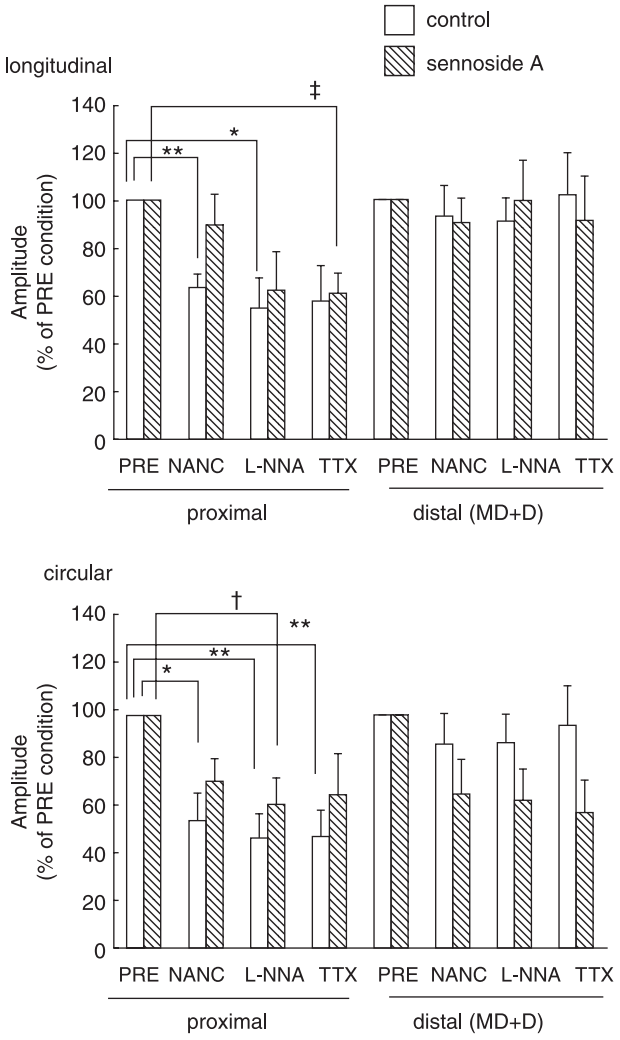

Fig. 5. Effect of non-adrenergic, non-cholinergic (NANC), N-ω-nitro-L-arginine (L-NNA) and tetrodotoxin (TTX) on sennoside A-induced changes in spontaneous contractions. In the control group, NANC treatment decreased the amplitude of longitudinal and circular contractions in the proximal colon, but not in the distal colon. In the sennoside A group, this treatment only slightly depressed the amplitude of contractions in the proximal colon. Values are given as mean ± S.E.M. (control, n = 4–8; sennoside A, n = 4–9). *P < 0.05 and **P < 0.01, significantly different from pretreatment condition (PRE) in control group by the paired t-test. †P < 0.05 and ‡P < 0.01, significantly different from PRE in the sennoside A group by the paired t-test. NANC condition obtained using 1 μM atropine sulfate, 1 μM phentolamine and 1 μM propranolol; L-NNA condition obtained using 0.1 mM N-ω-nitro-L-arginine; TTX condition obtained using 0.1 μM tetrodotoxin.

these effects are associated closely with luminal prostanoid level and only partially with cholinergic nerve mediation.

Sennosides behave as pro-drugs which, upon arrival in the large intestine, are broken down by bacterial activity to release active metabolites [3–6]. Previous studies on dogs [12], cats [19] and rats [13] using implanted strain gauges have suggested that senna glycosides act to stimulate propulsive patterns and inhibit segmental mixing movements. These effects were considered to alter, rather than increase, the spiking activity in the colon, leading to excretion of faeces.

Parts of the digestive tract from oesophagus to rectum display particular functions. In the colon, the inherent functions are to absorb water from luminal contents and to retain and expel the residue. Two types of contractions are seen in rat colon, one characterized by high amplitude and low frequency, the other by low amplitude and high frequency [20]. At the proximal colon, high-amplitude low-frequency contractions of the circular muscle are very active and probably related to the mixing and retention of luminal contents to allow water absorption. Conversely, low-amplitude high-frequency contractions of the circular muscle in the proximal colon are suggested to be anti-peristaltic in nature, again contributing to the retention of contents [17].

In the present study, sennoside A significantly diminished the amplitude of circular muscle contractions > 1 cm H_2O pressure in the proximal colon, but did not change the number of contractile events. This decrease was considered to result from a reduction in event number (frequency) of high-amplitude contractions in the proximal colon. Accordingly, sennoside A was suggested to primarily depress the development of high-amplitude contractions of circular muscle in the proximal colon. Together with previously reported findings, high-amplitude contractions of circular muscle in the proximal colon probably correspond to high-amplitude low-frequency contractions observed in the rat colon [20]. Sennoside A was thus considered to result in purgative actions in the proximal colon, because this agent shortens the passage time through the proximal colon, resulting in insufficient absorption of water. This finding is consistent with previous reports of sennoside A inducing depression of segmental mixing movements. In the distal colon, however, sennoside A produced a significant increase in amplitude of longitudinal and circular muscle contractions, suggesting that this type of movement will accelerate the transit of colonic contents. Significant changes after administration of

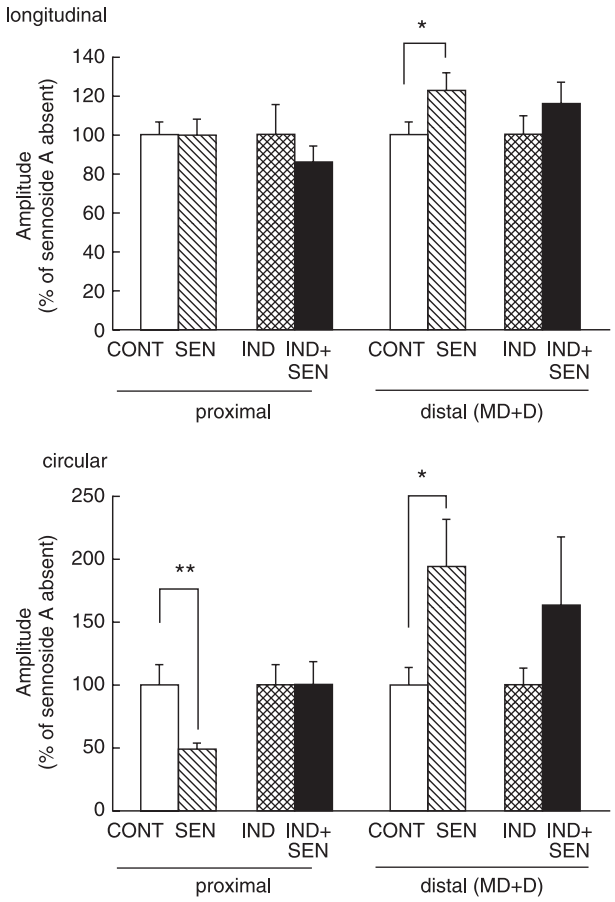

Fig. 6. Effect of indomethacin on sennoside A-induced changes in spontaneous contractions. Significant differences in amplitude between the control group (CONT; n = 21–26) and sennoside A (SEN; n = 23–30) group disappeared in mice treated with indomethacin (IND; n = 4–8) or indomethacin in combination with sennnoside A (IND + SEN; n = 6–12). Values are given as mean ± S.E.M. *P < 0.05 and **P < 0.01, significantly different from CONT by the Aspin-Welch t-test.

sennoside A were attenuated in mice recovering from diarrhoea 16 hr after application, suggesting that these changes were intimately responsible for purgative action. In contrast, sennoside A did not modify the movements in the mid-proximal colon.

The effects of autonomic nerve control on sennoside A-induced colonic motility were assessed by means of NANC condition, N-ω-nitro-L-arginine and tetrodotoxin. In the proximal colon of the control group, contractions of longitudinal and circular muscles were moderately but significantly lowered by NANC condition, suggesting cholinergic involvement in spontaneous movement. In the sennoside A group, the effects of NANC condition were greatly reduced, suggesting that sennoside A at least partly prevents the spontaneous motility due to cholinergic mediation. However, neither nitrergic nor presynaptic neuronal mechanisms were involved in control of spontaneous motility after treatment with NANC condition. In the distal colon, cholinergic participation was considered scarce.

The laxative effect induced by anthranoids has been attributed to two independent mechanisms, namely changes in colonic motility leading to accelerated large intestinal transit [4,9,10], and alterations in colonic absorption and secretion resulting in fluid accumulation [7–9]. The secretory effects of sennosides have been shown to be mediated through prostaglandin functions, because these effects are blocked by indomethacin [15,21], and as in intestinal transit, prostaglandins may also be involved in the mechanism of action of anthranoid laxatives [10,11]. In the present experiment, a purgative action appeared in all mice treated with sennoside A, compared to only four of nine mice treated with a combination of indomethacin and sennoside A. When the colon was isolated from mice, more voluminous and muddy stools were observed in indomethacin-treated mice with or without sennoside A. These findings, along with previous results, suggest that the effects of indomethacin on sennoside A-induced spontaneous motility occur in the proximal and distal colon. As a result, indomethacin attenuated the decrease in amplitude of circular muscle motility in the proximal colon. Likewise, the significant increase in the amplitude of longitudinal and circular muscle motility in the distal colon disappeared after treatment with indomethacin. The effects of sennoside A in the proximal and distal colon are thus considered to be closely related to prostaglandin mediation, both in controlling secretion and motility, as also suggested by previous reports.

In conclusion, sennoside A produces regionally differential effects on spontaneous colonic contractions in mice, and these effects are related only slightly to cholinergic nerve mediation, but much more to prostaglandin mechanisms. It is likely that sennoside A inhibits circular muscle contractions in the proximal colon, leading to shortening of the passage time of luminal contents and insufficient absorption of water, and thus accelerates the transit of luminal contents in the distal colon.

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