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Electroacupuncture at ST-36 accelerates colonic motility and transit in freely moving conscious rats

Masahiro Iwa,[1,2] Megumi Matsushima,[4] Yukiomi Nakade,[1] Theodore N. Pappas,[1] Mineko Fujimiya,[3] and Toku Takahashi[1]

[1]*Department of Surgery, Duke University and Durham Veterans Affairs Medical Center, Durham, North Carolina; and* [2]*Department of Health Promoting Acupuncture and Moxibustion, Meiji University of Oriental Medicine, Kyoto;* [3]*Department of Anatomy, Shiga University of Medical Science, Shiga; and* [4]*Department of Surgery, Meiji University of Oriental Medicine, Kyoto, Japan*

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Iwa, Masahiro, Megumi Matsushima, Yukiomi Nakade, Theodore N. Pappas, Mineko Fujimiya, and Toku Takahashi. Electroacupuncture at ST-36 accelerates colonic motility and transit in freely moving conscious rats. *Am J Physiol Gastrointest Liver Physiol* 290: G285–G292, 2006. First published October 27, 2005; doi:10.1152/ajpgi.00068.2005.—Acupuncture is useful for functional bowel diseases, such as constipation and diarrhea. However, the mechanisms of beneficial effects of acupuncture on colonic function have scarcely ever been investigated. We tested the hypothesis that electroacupuncture (EA) at ST-36 stimulates colonic motility and transit via a parasympathetic pathway in conscious rats. Hook-shaped needles were inserted at bilateral ST-36 (lower limb) or BL-21 (back) and electrically stimulated at 10 Hz for 20 min. We also studied c-Fos expression in response to EA at ST-36 in Barrington's nucleus of the pons. EA at ST-36, but not BL-21, significantly increased the amplitude of motility at the distal colon. The calculated motility index of the distal colon increased to132 ± 9.9% of basal levels ($n = 14$, $P < 0.05$). In contrast, EA at ST-36 had no stimulatory effects in the proximal colon. EA at ST-36 significantly accelerated colonic transit [geometric center (GC) = 6.76 ± 0.42, $n = 9$, $P < 0.001$] compared with EA at BL-21 (GC = 5.23 ± 0.39, $n = 7$). The stimulatory effect of EA at ST-36 on colonic motility and transit was abolished by pretreatment with atropine. EA-induced acceleration of colonic transit was also abolished by extrinsic nerve denervation of the distal colon (GC = 4.69 ± 0.33, $n = 6$). The number of c-Fos-immunopositive cells at Barrington's nucleus significantly increased in response to EA at ST-36 to 8.1 ± 1.1 cells/section compared with that of controls (2.4 ± 0.5 cells/section, $n = 3$, $P < 0.01$). It is concluded that EA at ST-36 stimulates distal colonic motility and accelerates colonic transit via a sacral parasympathetic efferent pathway (pelvic nerve). Barrington's nucleus plays an important role in mediating EA-induced distal colonic motility in conscious rats.

Barrington's nucleus; c-Fos; pelvic nerve

ACUPUNCTURE IS EMPIRICAL MEDICINE that has been used in China for more than 3,000 years (7, 20). Acupuncture medicine has been used in clinical practice in Japan as well for over 1,500 years. In recent years, acupuncture has been rapidly accepted widely throughout the world including the United States. In November 1997, the National Institutes of Health (NIH) held a consensus conference regarding acupuncture and issued a statement stating that acupuncture is effective treatment for nausea and vomiting postoperatively and associated with chemotherapy (19, 32).

Acupuncture has been used for treating functional gastrointestinal (GI) disorders, including irritable bowel syndrome (8, 15, 25), functional dyspepsia (13), constipation (4, 9, 23, 55), and diarrhea (2, 5, 43). However, the precise mechanism of acupuncture on GI motility remains to be clarified.

ST-36 is one of the most commonly used acupoints for GI diseases. ST-36 is located at the proximal one-fifth of the craniolateral surface of the leg distal to the head of the tibia in a depression between the muscles of the cranial tibia and long digital extensor.

In recent years, numerous studies have demonstrated the mechanisms of acupuncture on gastric motility and emptying. From our laboratory, Tada et al. (45) showed that manual acupuncture on the right abdomen induces gastric relaxations in anesthetized rats, which are mediated via a sympathetic nerve and ventrolateral medulla (VLM) of the brain stem. Tatewaki et al. (48) demonstrated that manual acupuncture at ST-36 induces dual effects, either stimulatory or inhibitory, on gastric motility in conscious rats. The stimulatory effects are mediated via vagal efferent and opioid pathways. Opioids may serve the long-lasting stimulatory effects of acupuncture on gastric motility (48).

Electroacupuncture (EA) is a combined procedure with acupuncture and electrical current stimulation instead of manual manipulations of needles. EA is more frequently used in clinical and research setting in recent years. Ouyang et al. (38) demonstrated that EA at PC-6 (Neiguan, wrist) and ST-36 accelerates gastric emptying of liquid in dogs via a vagal pathway. Moreover, EA at ST-36 can restore impaired gastric accommodation in vagotomized dogs probably via a sympathetic pathway (37).

Despite its well-established effects on gastric motility, the effects of acupuncture on colonic motility have scarcely ever been investigated. The purpose of this study was to investigate whether EA affects colonic motility and transit in conscious rats and to clarify the mechanisms of stimulatory effects of EA on colonic motility.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats weighing 250–300 g were purchased and maintained on a 12:12-h light-dark cycle (0800–2000) with free access to food and water. All animals were kept in individual cages in a controlled environment with constant temperature (23°C).

Address for reprint requests and other correspondence: T. Takahashi, Surgical Service 112, VA Medical Center, 508 Fulton St., Durham, NC 27705 (e-mail: ttakahas@duke.edu).

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All animal experiments were carried out in accordance with the NIH *Guide for the Care and Use of Laboratory Animals* and approved by the Institutional Animal Care and Use Committee of Durham Veterans Affairs Medical Center.

Recording of proximal and distal colonic motility in response to EA in conscious rats. Rats were fasted overnight and anesthetized with an intraperitoneal injection of pentobarbital sodium (50 mg/kg). Through a midline laparotomy, two strain-gauge transducers were implanted on the serosal surface of the proximal and distal colon to record circular muscle contractions, as previously described (21, 45, 48). The proximal strain gauge was placed ~2 cm distal to the ileocecal junction, and the distal strain gauge was placed ~5 cm proximal to the peritoneal reflection. The strain-gauge transducers were calibrated before the implantation surgery. The wires of the transducer were exteriorized from the abdominal cavity and subcutaneously tunneled between the shoulder blades, where a small skin incision was made. After the abdominal skin was sutured, the wires were covered by a rat protective jacket. Rats were allowed to recover from the surgery for 7 days.

Seven days after the surgery, recording of colonic motility was performed. After an overnight fast, the wires from the transducers were connected to the recording system (Power Lab, model 8SP, ADInstruments; Colorado Springs, CO). Colonic motility was recorded at least 1 h before EA.

To exclude the influence of anesthesia and restraint conditions, rats were allowed to move freely in the cage during the motility recording and EA procedure. To avoid the spontaneous removal of inserted acupuncture needles from the rat body, we invented hook-shaped needles instead of the usual straight acupuncture needles. Hook-shaped needles (0.25 mm in diameter) were made from the suture needles (6-0 Prolene-taper; Somerville, NJ; Fig. 1) and were soldered to an electric cord. Hook-shaped needles were inserted to a depth of 5 mm into the skin and underlying muscles at either ST-36 or BL-21 (Weishu; back) bilaterally. ST-36 is located at 5 mm lateral and lower from the anterior tubercle of the tibia in rats (47). BL-21, used as a sham acupoint, is located at 5 mm lateral of the spinous process of the 12th thoracic vertebrae in rats (53). Needles were inserted into the acupoints, which were stimulated by electrical square pulse width (1 mA) using an electrical stimulator (SEN-3201, Nihonkoden; Tokyo, Japan). The output of the device was checked by the analog multimeter (GMT-18A, GB Instruments; North Canton, OH) before EA. After 1 h of baseline recording, EA was performed for 20 min. After EA was finished, needles were removed, and the recording of colonic motility was continued for 5 h.

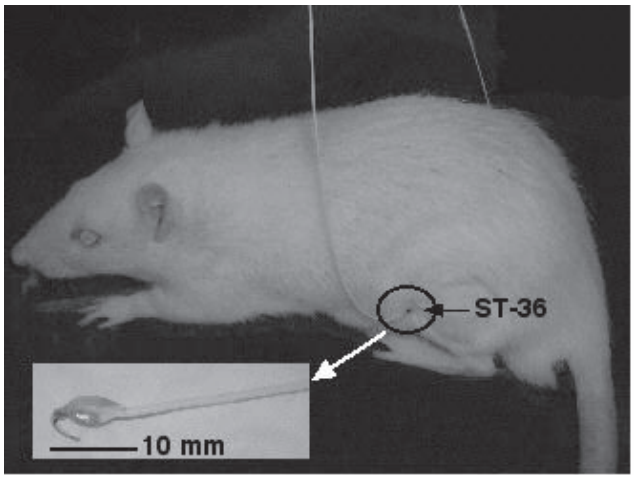

Fig. 1. New procedure for electroacupuncture (EA) at ST-36 in a freely moving conscious rat. A hook-shaped needle with an attached electrical cord was inserted at acupoint ST-36 to perform EA. Rats were allowed to move freely in the cage during EA.

To investigate whether the cholinergic pathway is involved in mediating the stimulatory effects of EA on distal colonic motility, atropine (50 μg/kg) (17, 48) was intramuscularly administered 70 min before EA. Saline (0.3 ml)-injected rats served as controls.

Experiments of colonic transit in response to EA at ST-36 in conscious rats. Rats were anesthetized with pentobarbital sodium (50 mg/kg) intraperitoneally. Through a midline laparotomy, an indwelling Silastic cannula was inserted into the cecum and positioned to enter the proximal colon to administer a nonabsorbable radioactive marker (^{51}Cr), as previously reported (17). The cannula was run under the skin to an opening made between the shoulder blades. The abdominal wall was closed, and rats were allowed to recover from the surgery for 7 days.

Seven days after the surgery, colonic transit experiments were performed. Rats were fasted overnight. Saline (0.3 ml) was administered intramuscularly. After 10 min, EA at ST-36 or BL-21 acupoints was performed for 20 min. Immediately after EA finished, $Na^{51}CrO_4$ (0.5 μCi, in 0.2 ml saline) was instilled into the proximal colon through the cannula. Three hours afterward, rats were euthanized by isoflurane inhalation, and the entire colon was surgically removed and divided into 10 equal segments. Each segment was placed into a test tube, and radioactivity was counted by a gamma counter for 1 min. The geometric center (GC) of the distribution of ^{51}Cr within the colon is the center of gravity for the distribution of radiochromium, and it was calculated using the following equation, as previously described (17): GC = Σ (fraction of ^{51}Cr per segment × segment number).

To investigate whether the cholinergic pathway is involved in mediating the stimulatory effects of EA at ST-36 on colonic transit, atropine (50 μg/kg) was intramuscularly administered 60 min before EA. To investigate whether endogenous opioids are involved in mediating the stimulatory effects of EA on colonic transit, naloxone (a nonspecific opioid receptor antagonist, 1 mg/kg) was administered intramuscularly 10 min before EA.

To investigate the possible involvement of extrinsic nerves in mediating EA-stimulated colonic transit, the descending fibers from around the caudal mesenteric artery and the ascending fibers from the pelvic plexus were transected by dissecting all tissues around the vessels. The peritoneal reflection was also dissected. The inferior mesenteric artery and marginal artery were preserved. Sham-operated rats received a simple manipulation of the distal colon.

c-Fos immunohistochemistry. To investigate the possible neural pathway mediating EA-induced colonic motor responses, we performed c-Fos immunohistochemistry of the brain stem. Ninety minutes after the EA at ST-36, rats were anesthetized with pentobarbital sodium (50 mg/kg ip). As previously reported (45), rats were transcardially perfused for 10 min with 0.01 M PBS to wash out the blood and then perfused with a fixative (4% paraformaldehyde), 0.5% glutaraldehyde, and 0.2% picric acid in 0.1 M phosphate buffer at 4°C for 10 min at a speed of 30 ml/min. Rats that received no EA served as controls. The brain was removed from the skull, immersed for 24 h in the postfixative (4% paraformaldehyde and 0.2% picric acid in 0.1 M phosphate buffer) at 4°C for 1 wk. After being washed for 30 min with PBST, sections were incubated in biotinylated anti-rabbit IgG (Vector Laboratories; Burlingame, CA) diluted 1:1,000 in PBST at room temperature for 2 h. Sections were then washed and placed in avidin-biotin peroxidase complex (Elite, Vector Laboratories) diluted 1:2,000 in PBST for 1.5 h at room temperature. Immunoreactivity was visualized by an incubation with 0.05 M Tris·HCl buffer (pH 7.6) containing 0.01% diaminobenzidine, 1% ammonium nickel sulfate, and 0.0003% H_2O_2 for 30 min at room temperature. The stained sections were mounted on gelatin-coated glass slides, dehydrated with graded ethanol, and placed on a cover-slip with Entellan (Merck; Darmstadt, Germany).

For c-Fos immunohistochemistry of the dorsal motor nucleus of vagus (DMV) and Barrington's nucleus, coronal frozen sections (20 μm) of the brain were cut at the interaural levels of −4.68 to −4.8 mm and −0.8 to −1.04 mm, respectively, according to the atlas of

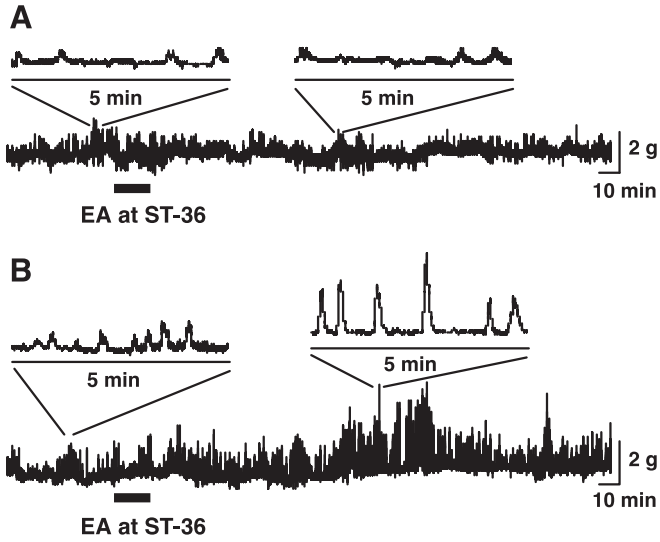

Fig. 2. Original tracings of colonic motility before and after EA at ST-36 of the proximal (P) colon (*A*) and distal (D) colon (*B*). Colonic motility was recorded for 1 h before EA and for 5 h after EA. EA at ST-36 increased colonic motility at the distal colon but not proximal colon. The stimulatory effect of EA on colonic motility was observed for over 3 h after the cessation of EA.

Paxinos and Watson (41). Under a light microscope at 100 × magnification, the total number of c-Fos-immunoreactive cells was counted in each brain nucleus. Mean values of each brain nucleus were determined by sampling from three randomly selected sections cut through the specific brain nuclei.

Statistical analysis. Quantification of colonic motility was studied by calculating the motility index (MI). The MI is equivalent to the area under the curve of motility recording. MI was calculated using a computer-assisted system (Power Lab, ADInstruments), as previously reported (21). MI was calculated every 30 min before EA. MI 60 min before EA was expressed as 100%, and the average MI 30 min before EA was calculated (basal MI). MI was similarly calculated every 30 min after EA for 5 h. The stimulated MI after EA was compared with the basal MI and expressed as the percent increase of MI in each rat. The comparison was performed at each point after EA using repeated-measures ANOVA.

In the colonic transit study, statistical comparisons among multiple groups were first made using one-way ANOVA, and, if differences were detected, post hoc analysis was then performed. Bonferroni and Dunnett tests were used to test the significance.

In the c-Fos expression study, statistical analysis was performed using the Student's *t*-test. Data are expressed as means ± SE.

For the frequency response study, four rats were used. Each rat received a different frequency of EA (1, 3, 10, and 30 Hz) 4–5 days apart. For the comparison between EA at ST-36 and BL-21, 11 rats were used. Each rat received EA (10 Hz) at ST-36 and BL-21 4–5 days apart. For the atropine study, six rats were used. Each rat received an injection of atropine and saline (control) before EA at ST-36 (10 Hz) 4–5 days apart.

Materials. Atropine and naloxone were obtained from Sigma Chemical (St. Louis, MO). Rats were purchased from Charles River Laboratories (Raleigh, NC).

RESULTS

Effects of EA on colonic motility in conscious rats. In 9 of 11 rats (82%), EA at ST-36 significantly stimulated motility of the distal colon. The remaining two rats (18%) showed no apparent motor responses to EA. Three of eleven rats (27%) slightly responded to EA at the proximal colon. The remaining eight rats (73%) showed no apparent motor responses to EA at the proximal colon.

The stimulatory effect of EA on distal colonic motility was maintained for over 3 h after EA finished. The peak amplitude of contractions increased from 1.98 ± 0.2 to 4.68 ± 0.3 g by EA ($n = 11$, $P < 0.01$ by paired *t*-test; Fig. 2).

MI was calculated with the sum of every 30-min period and was expressed as the percentage of change on the basis of baseline. In the distal colon, MI from 60 to 180 min after EA at-ST-36 significantly increased compared with MI before EA ($n = 11$, $P < 0.05$). In contrast, MI of the proximal colon did not significantly change after EA ($n = 11$; Fig. 3*A*).

Figure 3*B* shows that MI changes only in responded rats to EA (proximal colon, $n = 3$; distal colon, $n = 9$). MI of the distal colon from 60 to 180 min after EA significantly increased ($n = 9$, $P < 0.01$). Although MI slightly increased at 90 and 120 min after EA of the proximal colon, there was no statistically significance observed even in responders ($n = 3$; Fig. 3*B*).

EA at 10 Hz evoked a maximal contraction at the distal colon. In contrast, EA up to 30 Hz had no remarkable contractions in the proximal colon (Fig. 4).

EA at BL-21 (10 Hz) caused no significant effect on distal colonic motility. MI after EA at BL-21 did not increase for 5 h (Fig. 5*A*). EA at BL-21 (30 Hz) also had no significant effect on distal colonic motility (data not shown).

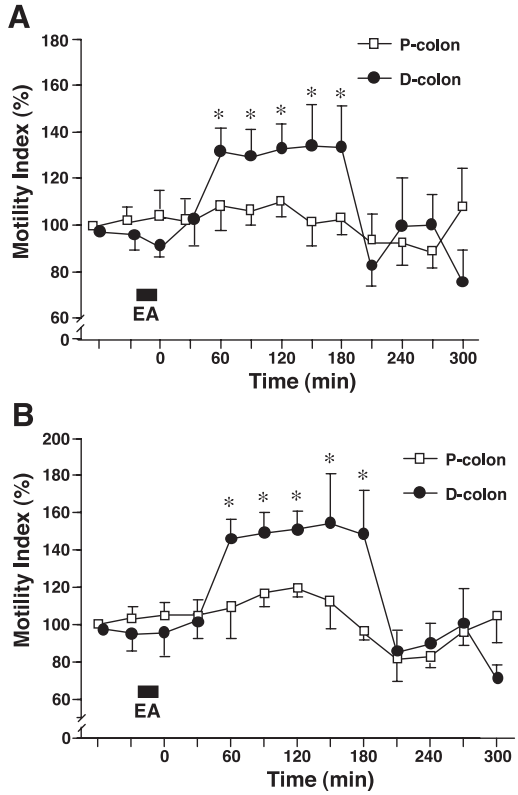

Fig. 3. *A*: motility index (MI) before and after EA at ST-36 (10 Hz) at the proximal colon ($n = 11$) and distal colon ($n = 11$). *B*: MI in responders to EA at the proximal colon ($n = 3$) and distal colon ($n = 9$). MI of the distal colon, but not the proximal colon, was significantly increased in response to EA for over 3 h ($n = 11$, $^*P < 0.05$ vs. before EA; *A*). MI of the proximal colon slightly increased at 90 and 120 min after EA, but there was no significant difference observed (*B*). MI is given as the percentage of baseline MI before EA.

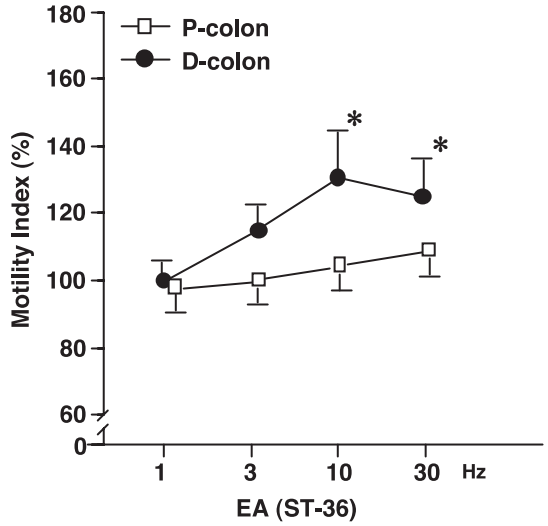

Fig. 4. MI calculated at 1 h after EA at ST-36 (1–30 Hz) of the proximal and distal colon. MI significantly increased 1 h after EA at ST-36 (10 and 30 Hz) at the distal colon but not proximal colon ($n = 4$, $^*P < 0.05$ vs. before EA). MI is given as the percentage of baseline MI before EA.

Atropine itself significantly attenuated spontaneous colonic motility within 30 min. Atropine completely abolished EA-stimulated colonic motility ($n = 6$, $P < 0.05$; Fig. 5*B*).

Effects of EA on colonic transit in conscious rats. EA at ST-36 significantly accelerated colonic transit (GC = 6.76 ± 0.42, $n = 9$, $P < 0.01$) compared with controls (GC = 5.23 ± 0.26, $n = 9$; Fig. 6*A*). On the other hand, EA at BL-21 had no significant effect on colonic transit (GC = 5.23 ± 0.39, $n = 7$).

GC in rats pretreated with atropine was 4.12 ± 0.25 ($n = 6$). EA at ST-36 had no more stimulatory effect on colonic transit in atropine-treated rats (GC = 4.32 ± 0.2, $n = 6$; Fig. 6*A*). After pretreatment with naloxone, the stimulatory effect of EA at ST-36 was still observed (GC = 6.38 ± 0.58, $n = 9$, $P < 0.05$).

GC in rats pretreated with sham operation was 5.34 ± 0.2 ($n = 6$). EA at ST-36 significantly accelerated colonic transit in sham-operated rats (GC = 6.77 ± 0.57, $n = 6$, $P < 0.01$; Fig. 6*B*). In contrast, EA at ST-36 had no more stimulatory effect on colonic transit in rats treated with extrinsic nerve denervation (GC = 4.69 ± 0.33, $n = 6$; Fig. 6*B*).

Effects of EA at ST-36 on c-Fos expression in the brain stem. To investigate whether the pons and medulla are involved in mediating EA-induced colonic motility, we studied c-Fos expression in the Barrington's nucleus and DMV in response to EA.

The number of c-Fos-immunopositive cells at the Barrington's nucleus significantly increased in response to EA to 8.1 ± 1.1 cells/section compared with that of controls (2.4 ± 0.5 cells/section, $n = 3$, $P < 0.01$ by Student's *t*-test; Fig. 7).

The number of c-Fos-immunopositive cells at the DMV also significantly increased in response to EA, to 8.1 ± 0.5 cells/section compared with that of controls (2.1 ± 0.4 cells/section, $n = 3$, $P < 0.001$ by Student's *t*-test). Moreover, the number of c-Fos-immunopositive cells at the medial portion of the DMV significantly increased in response to EA to 6.8 ± 0.4 cells/section compared with that of the lateral portion (1.3 ± 0.2 cells/section, $n = 3$, $P < 0.0001$ by Student's *t*-test; Fig. 8). EA at ST-36 did not increase the number of c-Fos-immunopositive cells at the lateral portion. Therefore, c-Fos-immunopositive cells in response to EA were dense at the medial portion of the DMV.

The number of c-Fos-immunopositive cells at NTS also significantly increased in response to EA (to 16.8 ± 2.5 cells/section) compared with controls (3.2 ± 1.6 cells/section, $n = 3$, $P < 0.05$ by Student's *t*-test).

DISCUSSION

In experimental animals, especially rodents, the acupuncture procedure has been performed in anesthetized conditions or restrained conditions if animals are unanesthetized. Anesthetic agents may interfere with the possible neural pathway. Various anesthetic agents (pentobarbital, ketamine, chloral hydrate, urethane, and metofane) eliminate evoked firing and suppressed spontaneous firing in the central nervous system (CNS) (52). Anesthesia upregulates c-Fos expression in the CNS (11, 46). It is highly possible that acupuncture affects GI functions via the peripheral nervous system and CNS. Anesthetic agents may interfere with the neural activation induced by acupuncture. Therefore, it is preferable that GI function studies are performed without any anesthetic agents.

Colonic transit and fecal expulsion are stimulated by a variety of acute stresses (18, 33, 44, 54). Restraint stress upregulates c-Fos expression in the CNS (12, 31). It is possible that immobilization affects several physiological parameters and may mask specific acupuncture responses.

In more than 80% of rats tested, EA at ST-36, but not BL-21, stimulated motility in the distal colon, whereas EA had no stimulatory effect in the proximal colon in more than 70% of rats tested. Although 27% of rats slightly responded to EA in

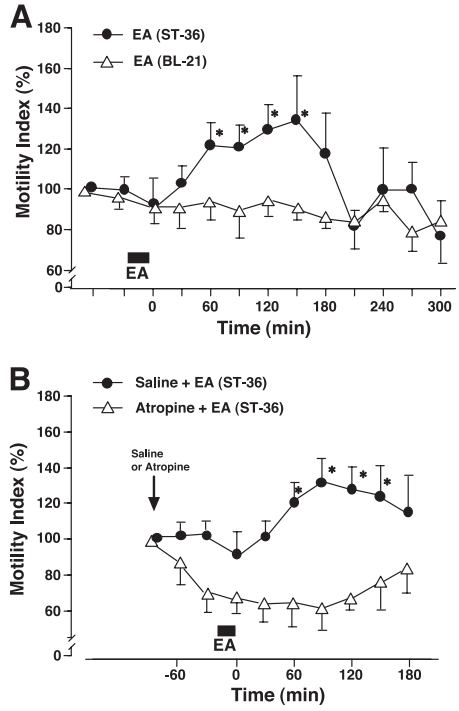

Fig. 5. *A*: effects of EA (10 Hz) at ST-36 and BL-21 on distal colonic motility. EA at BL-21 did not significantly affect colonic motility ($n = 6$). *B*: effects of atropine on EA at ST-36-stimulated distal colonic motility. Atropine itself significantly attenuated spontaneous colonic motility within 30 min. Atropine completely abolished EA at ST-36-stimulated colonic motility ($n = 6$, $^*P < 0.05$ vs. before EA).

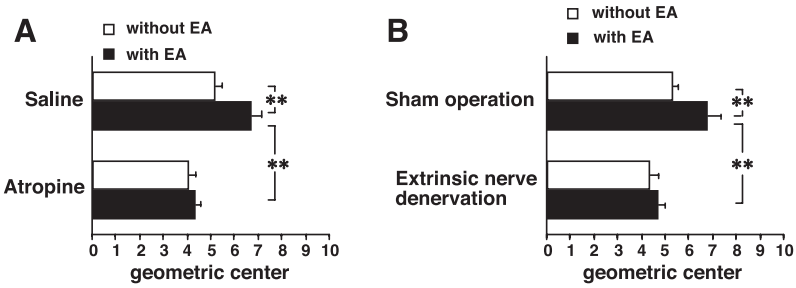

Fig. 6. Effects of atropine (*A*) and extrinsic nerve denervation (*B*) on accelerated colonic transit induced by EA at ST-36. EA at ST-36 significantly accelerated colonic transit compared with controls ($n = 9$, $^{**}P < 0.01$ vs. controls). Atropine abolished EA-stimulated colonic transit ($n = 6$, $^{**}P < 0.01$ vs. the EA group). In sham-operated rats, EA at ST-36 significantly accelerated colonic transit ($n = 6$, $^{**}P < 0.01$ vs. the sham-operated without EA group). Extrinsic nerve denervation abolished EA-stimulated colonic transit ($n = 6$, $^{**}P < 0.01$ vs. the EA group).

the proximal colon, there was no statistically significance observed in these responders. In the total of 11 rats, MI significantly increased in response to EA at the distal colon. These results suggest that EA at ST-36 stimulates distal colonic motility but not proximal colonic motility.

It is well known that physical discomfort/stress stimulates colonic motility (44). Altered colonic motility may result from physical discomfort/stress experienced by the rat when stimulated with EA at ST-36 but not at BL-21. Because a higher frequency of EA may cause physical discomfort/stress, we tested EA at BL-21 up to 30 Hz. However, EA at BL-21 (30 Hz) had no remarkable effect on colonic motility. Therefore, it is unlikely that the nonresponse to EA at BL-21 is due to the fact that BL-21 is less stressful than that of ST-36. We cannot exclude the possibility that the nonresponse to EA at BL-21 is due to the fact that BL-21 is physically further away from the colon and its extrinsic innervation.

In the clinical practice of acupuncture, it is well known that effects of acupuncture vary among individuals. The underlying mechanisms responsible for the differences between responders and nonresponders to acupuncture remain to be clarified. Lee et al. (27) demonstrated that cholecystokinin-A receptor expression in the hypothalamus is significantly higher in nonresponders than in responders in EA-mediated analgesic effects (27). The differences between responders and nonresponders to EA may depend on neuronal activity in the CNS.

We further investigated the neural mechanisms of stimulatory effects of EA on distal colonic motility and colonic transit. The stimulatory effects of EA on distal colonic motility and colonic transit were abolished by pretreatment with atropine, suggesting that EA-induced distal colonic motility and colonic transit are mediated via muscarinic receptors.

Parasympathetic efferent innervation of the colon involves the vagus nerve and pelvic nerve. Vagal innervation of the cecum is dense, and the density of vagal innervation becomes rapidly much more sparse distally toward the rectum in rats (3). In contrast, the pelvic nerve innervates the distal colon and rectum (29, 34) but not the proximal colon in rats. Our study suggested that EA at ST-36 stimulates pelvic efferents resulting in contractions of the distal colon in rats.

In this study, we dissected the mesocolon around the distal colon and peritoneal reflection and stripped the surrounding tissues around the distal colon at the peritoneal reflection. Branches of the pelvic nerves (the rectal nerves) of rats enters into the gut at the peritoneal reflection and ascends up to the middle colon (29, 34). The pelvic efferent and afferent fibers ascend in the colonic wall up to the proximal part of the descending colon (34).

Colonic transit was not accelerated by EA in rats treated with extrinsic nerve denervation. These results suggest that EA at ST-36 accelerates distal colonic motility and colonic transit via a parasympathetic pelvic nerve.

Barrington's nucleus has long been considered solely as the pontine micturition center. Selective lesion of Barrington's nucleus disturbs the micturition reflex (24). Conversely, electrical or chemical stimulation of Barrington's nucleus induces bladder contractions (35, 39). The role of Barrington's nucleus in mediating colonic function has been revealed in recent years

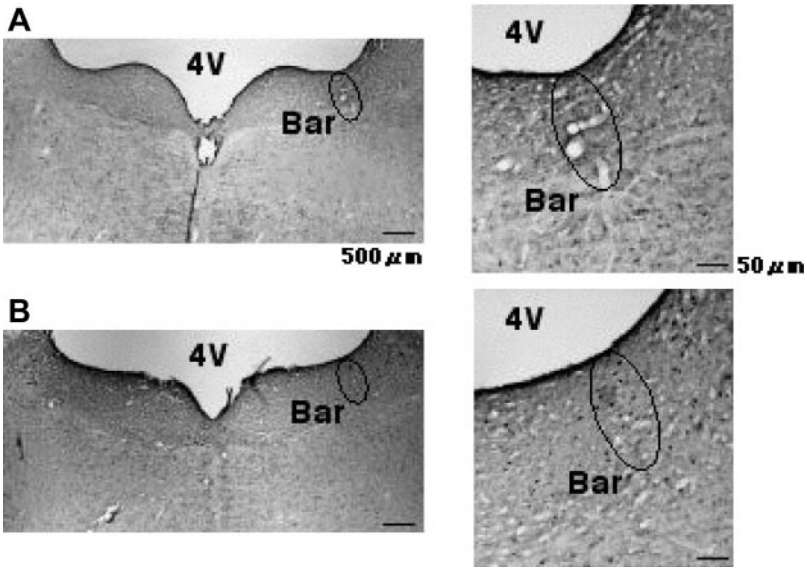

Fig. 7. c-Fos expression at Barrington's nucleus (Bar) in control rats (*A*) and EA-treated rats (*B*). The number of c-Fos-immunopositive cells significantly increased in response to EA to 8.1 ± 1.1 cells/section compared with that of controls (2.4 ± 0.5 cells/section, $n = 3$, $P < 0.01$ by Student's *t*-test). 4V, fourth ventricle.

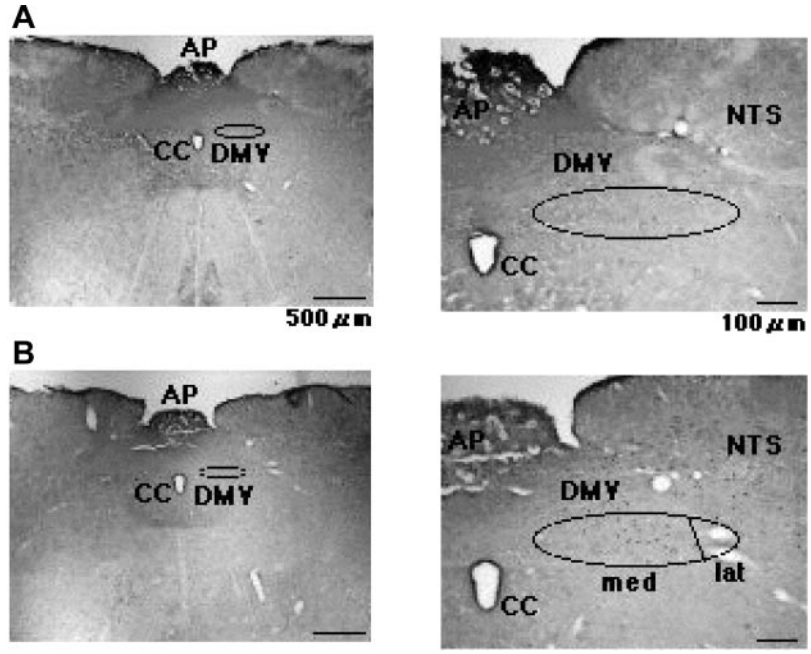

Fig. 8. c-Fos expression at the dorsal motor nucleus of the vagus (DMV) in control rats (*A*) and EA-treated rats (*B*). The number of c-Fos-immunopositive cells at the DMV significantly increased in response to EA to 8.1 ± 0.5 cells/section compared with controls (2.1 ± 0.4 cells/section, $n = 3$, $P < 0.001$ by Student's *t*-test). Especially, c-Fos-immunopositive cells were dense at the medial portion of the DMV. AP, area postrema; CC, central canal; NTS, nucleus of the solitary tract; med, medial; lat, lateral.

(40, 51). Pavcovich et al. (40) demonstrated that neurons at Barrington's nucleus were transsynaptically labeled from the distal colon. Selective chemical activation of Barrington's nucleus increases intraluminal pressure of the distal colon (40), which was antagonized by a muscarinic antagonist, scopolamine, and an intrathecal injection of a local anesthetic, lidocaine. These results suggest that Barrington's nucleus projects to the peripheral parasympathetic nerve (pelvic nerve).

In the present study, the number of c-Fos-immunopositive cells at Barrington's nucleus significantly increased by EA at ST-36 compared with controls. This result suggests that Barrington's nucleus plays an important role in mediating EA-induced distal colonic motility in conscious rats.

It has been shown that the stimulatory effect of acupuncture on gastric motility is abolished by spinal cord transection (42, 45). This suggests that impulses from sensory nerves activated by acupuncture travel to the brain through the spinal cord. From the results of the present study, we are not able to conclude whether EA-stimulated colonic motility is mediated via a spinal or supraspinal pathway. However, based on previous studies, it is conceivable that impulses from sensory nerves stimulated by EA travel to the pons through the spinal cord, resulting in activation of Barrington's nucleus.

Acupuncture at ST-36 stimulates gastric motility, and this stimulatory effect is mediated via the vagal nerve in conscious rats (48). Neurons at the DMV were transsynaptically labeled from the stomach and acupoint of ST-36 (26). These results suggest that the DMV plays an important role in mediating acupuncture-induced gastric motility.

In the present study, EA at ST-36 did not affect proximal colonic motility despite the fact that EA at ST-36 stimulates vagal efferents (48). Therefore, we also performed c-Fos immunohistochemistry at the DMV in rats treated with EA at ST-36. As a result, the number of c-Fos-immunopositive cells at the DMV was significantly increased by EA at ST-36 compared with that of controls. Especially, c-Fos-immunopositive cells were highly expressed in the medial portion of the DMV in response to EA.

The present study showed that the number of c-Fos-immunopositive cells at the nucleus of the solitary tract (NTS) also significantly increased in response to acupuncture. We (45) have previously showed that acupuncture significantly increased the number of c-Fos-immunopositive cells at the NTS. The NTS is well known to receive visceral sensory inputs. A recent study (50) has shown that NTS neurons also receive input from cutaneous mechanoreceptors and respond to activation of mechanosensitive and metabosensitive endings in skeletal muscle.

Vagal nerves are divided into three major divisions: gastric, celiac, and hepatic branches. The stomach is innervated by gastric branches, and the colon is innervated by celiac branches. In rats, the medial two-thirds of the DMV nucleus correspond to gastric branches and the lateral pole of the DMV corresponds to celiac branches (16). Once the retrograde tracer was injected into the colon, in all areas of the colon except the rectum, motoneuronal labeling was limited to the lateral third of the DMV (1). In contrast, gastric injections showed that motoneuronal labeling was limited to the medial portion of the DMV (1). Others (36) have reported that gastric efferent neurons are concentrated in the central portion of the DMV and project to the stomach via gastric branches. These results suggest that gastric branches of the vagal nerve project from the medial part of the DMV to the stomach and that celiac branches project from the lateral part of the DMV to the colon.

We showed that the number of c-Fos-immunopositive cells at the medial portion of the DMV significantly increased in response to EA. In contrast, EA did not alter the number of c-Fos-immunopositive cells at the lateral portion of the DMV. The present study suggests that EA stimulated the gastric branch, but not the celiac branch, of the vagus. Therefore, it seems unlikely that the EA-evoked increase in distal colonic motility was mediated by the vagus nerve.

The stimulatory effects of EA at ST-36 on distal colonic motility persisted for over 3 h. EA at ST-36 activates gastric myoelectric activity via vagal efferent pathways, and the excitatory effect of EA persisted for 90 min after cessation of EA (28). We (48) have recently demonstrated that the long-lasting stimulatory effects on gastric motility induced by acupuncture are mediated via opioid pathway. It is also known that naloxone antagonizes the antiemetic effects (49) and analgesic effects (10) of acupuncture.

Peripheral administration of opioids inhibits intestinal motility via stimulating gut opioid receptors (6, 22, 30). In contrast, intracerebroventricular administration of morphine stimulated colonic motility in the conscious dog (14). To study whether the long-lasting effect of acupuncture on colonic motility is mediated via a central opioid pathway, we investigated the effects of naloxone on EA-stimulated colonic transit. Pretreatment with naloxone did not antagonize the stimulatory effects of EA on colonic transit. This suggests that endogenous opioids are not involved in mediating EA-induced acceleration of colonic transit. Further study is needed to clarify the long-lasting effects of acupuncture in mediating colonic motility.

In summary, EA at ST-36 stimulated motility for over 3 h at the distal colon but not the proximal colon in freely moving conscious rats. EA at ST-36 also accelerated colonic transit, which was abolished by atropine and extrinsic nerve denervation. EA at ST-36 significantly increased c-Fos expression at Barrington's nucleus. It is suggested that the stimulatory effects of EA at ST-36 on distal colonic motility are mediated via a sacral parasympathetic efferent (pelvic nerve) that are projects from Barrington's nucleus of the dorsal pons. The verification of this neural pathway may contribute to understand the mechanisms of beneficial effects of acupuncture on functional bowel diseases.

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