Dual effects of acupuncture on gastric motility in conscious rats

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It has been demonstrated that the somatosensory inputs from the skin and/or muscle are involved in the control of various autonomic functions (22, 26). A series of investigations regarding somato-autonomic reflexes has also been carried out focusing on GI function. In some of those investigations, there is good evidence indicating the importance of cutaneo-sensory inputs in the autonomic control of GI motility. In anesthetized rats, for instance, it has been shown that the cutaneo-gastric reflexes mediate the inhibition and the stimulation of gastric motility via sympathetic and parasympathetic efferents, respectively (24, 28, 47). It was shown that the cutaneo-sensory stimulation induced by pinching abdominal skin of rats inhibits gastric motility by increasing sympathetic activity. On the other hand, cutaneo-sensory stimulation induced by pinching the hindlimb enhances gastric motility by increasing vagal activity (24). Although the mechanism(s) of acupuncture still remains mysterious, it could be possible to treat gastric dysmotility with an appropriate cutaneo-sensory stimulation.

It is generally believed that acupuncture at different points (acupoints) shows different effects. Previous studies suggest the site-specific inhibitory or stimulatory effects of acupuncture on gastric motility (24, 48). The pericardium (PC)-6 (Neiguan) at wrist and stomach (ST)-36 (Zusanli) at hindlimb are the common acupoints used for treating gastric symptoms such as nausea and vomiting (1, 35), suggesting that acupuncture at these acupoints may stimulate gastric motility. In contrast, acupuncture on the abdomen has been used for treating abdominal pain (8, 11, 17), suggesting that acupuncture at this point may inhibit gastric motility and/or reduce gastropasm.

Recently, the effects of acupuncture on GI function were mostly investigated with electric stimulation (electroacupuncture) (5, 41, 45, 46, 56). Electroacupuncture at ST-36 and PC-6 has been shown to enhance the gastric migrating motor complex (MMC) in conscious dogs (45). In addition, it has also been shown in conscious dogs that electroacupuncture accelerates...

ACUPUNCTURE HAS BEEN USED empirically in clinical practice in China for several millennia (59). In November 1997, the National Institutes of Health conducted a consensus conference regarding acupuncture and concluded that acupuncture is an effective treatment for several medical conditions. Although a large number of previous clinical studies support the efficacy of acupuncture for treating gastrointestinal (GI) symptoms and/or diseases (9, 16), little is known about the underlying mechanism(s).
liquid gastric emptying by stimulating vagal activity, improving gastric slow-wave rhythm, and enhancing antral contractile activity (41).

During over 5,000 years of history of traditional Chinese medicine, however, acupuncture had been used without electric stimulation until the discovery of electricity. Manual acupuncture was used during this period. It has been shown that manual acupuncture on the abdomen inhibits gastric motility in anesthetized rats (48). We recently showed in anesthetized rats that manual acupuncture at the lower abdomen induced gastric relaxations via the somato-sympathetic pathway (49). In conscious rats, however, it remains to be elucidated whether manual acupuncture at ST-36 affects gastric motility.

Although little is known about the mechanism(s) of acupuncture, recent investigations have suggested that the effects of electroacupuncture are mediated via the opioid system (6, 56). With our conscious rat model (21), therefore, we investigated whether manual acupuncture at ST-36 affects gastric motility.

**Materials and Methods**

Animal preparation. Male Sprague-Dawley rats (body wt 225–250 g) were maintained on a 12:12-h light-dark cycle (0800–2000) with free access to food and water. All animal experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee of Duke University and Durham Veterans Affairs Medical Center. The rats were fasted overnight and anesthetized with an intraperitoneal injection of pentobarbital sodium (45 mg/kg, Nembutal; Abbott Laboratories, N. Chicago, IL). Through a midline laparotomy, a strain gauge transducer was implanted on the serosal surface of the gastric antrum, as previously described (21, 49). Each transducer was calibrated before the surgery by 1- to 10-g weights. The wires from the transducer were exteriorized through the abdominal wall and subcutaneously tunneled between the shoulder blades, where a small skin incision was made. After being sutured to the skin, the wires were covered by a rat protective system (Star Medical, Tokyo, Japan). The rats were allowed to recover from the surgery for 7 days. During the period, the rats were trained to be restrained in a cage for at least 2 h every day until the day of acupuncture study. Our preliminary study showed that the fasting gastric motility was not significantly different between free-moving rats and restrained rats.

Recording of gastric motility in response to acupuncture in conscious rats. One week after the surgery (day 7 after surgery), the rats were fasted for 24 h with free access to water and then kept in restraint cages. The wires from the transducer were connected to the recording system (PowerLab model S8P, ADInstruments, Castle Hill, Australia), and the fasting gastric motor activity was recorded as a control for at least 1 h before the acupuncture. This control recording allowed us to identify two different types (type A and type B) of fasting gastric motor activity. In six rats (3 rats in each type), therefore, the fasting gastric motor pattern was examined again 3 days later (day 10 after surgery) to see whether the motor pattern changed.

After 1 h of recording, an acupuncture needle of 0.3 mm in diameter was inserted into the skin and underlying muscles at either ST-36 or BL-21 (Weishu). ST-36 is located on the stomach meridian, whereas BL-21, used as a control, is located on the urinary bladder meridian (7, 33). It was previously shown that ST-36 is located 5 mm lateral and lower from the anterior tubercle of the tibia in 200–250 g body wt (53). BL-21 is located 5 mm lateral of the spinous process of the 12th thoracic vertebrae (60). After being inserted at a depth of 5 mm at ST-36 or BL-21, as described in the literature (42), the needle was manipulated manually by twisting right and left once every second for 30 s. Then the acupuncture needle was immediately removed. The gastric motility was recorded during and after the acupuncture for 4 h. The same rats received manual acupuncture at either ST-36 or BL-21 on separate days, at least 7 days apart. All rats were finally examined at both acupoints.

To investigate whether the effects of acupuncture at ST-36 were reproducible in the same rats, six rats (3 rats for the stimulatory responses and 3 rats for the inhibitory responses) were examined again 1 wk later (day 14 after surgery).

Pharmacological and surgical treatments. After baseline experiments were finished, the rats were subsequently used in either pharmacological studies or surgical studies. Pharmacological studies, consisting of two independent series of experiments (atropine-hexamethonium-atropine methyl bromide and naloxone), were conducted. A total 14 rats (7 rats of type A for the stimulatory responses and 7 rats of type B for the inhibitory responses) were used for the atropine-hexamethonium-atropine methyl bromide experiments. To investigate whether the cholinergic pathway is involved in mediating the stimulatory effects of acupuncture on gastric motility, either atropine (a muscarinic receptor antagonist; 50 μg/kg) or hexamethonium (a nicotinic receptor antagonist; 20 mg/kg) was subcutaneously administered 60 min after the acupuncture. Four and three rats of each type were examined with atropine and hexamethonium, respectively. Our preliminary study showed that 50 μg/kg of atropine completely abolished bethanechol (40 μg/kg)-induced gastric contractions (50). Atropine blocks central cholinergic as well as peripheral cholinergic pathways. To investigate whether peripheral cholinergic pathways are involved in mediating acupuncture-induced gastric motility, atropine methyl bromide (a peripheral muscarinic receptor antagonist; 1 mg/kg sc) was used. Atropine methyl bromide was administered 60 min after the acupuncture on separate days in three rats that were used for the atropine study.

To investigate whether opioid is involved in mediating the stimulatory effects of acupuncture on gastric motility, naloxone (a nonspecific opioid receptor antagonist; 5 mg/kg ip) was administered 20 min before the acupuncture, as described in the literature (29). For the naloxone experiments, three rats of type A that showed the stimulatory responses to acupuncture were used. In the control study, saline was injected intraperitoneally 20 min before the acupuncture.

For the surgical treatment, seven rats (4 type A rats and 3 type B rats) were used. Their fasting gastric motor pattern and the responses to the acupuncture were already identified on day 14 after the first surgery. To investigate whether the vagal-cholinergic pathway is involved in the stimulatory or inhibitory effects of acupuncture on gastric motility, subtotal phragmatic bilateral truncal vagotomy was performed on day 15. One week after the vagotomy, acupuncture at ST-36 was performed again.

After the series of experiments was finished, rats were killed with pentobarbital sodium (200 mg/kg ip).
Analysis of motor activity. The fasting gastric motor activity was first analyzed visually to detect cyclic groupings of strong contractions (phase III-like contractions). Phase III-like contractions were defined as consisting of at least three potent contractions (>4 g of amplitude) with short duration (<5 min). Phase I-like contractions were defined by the long duration (>10 min) of silent phases consisting of small contractions (<2 g of amplitude). The rats were then classified into two groups: type A, showing no cyclic groupings of the phase III-like contractions; and type B, showing cyclic groupings of the phase III-like contractions. In the type A rats, the peak amplitude of contractions was calculated by averaging the highest peak values during each 10-min period for 60 min. In the type B rats, the peak amplitude was calculated by averaging the highest amplitude in each grouping of the phase III-like contractions during 60 min.

Quantification of gastric motility was also performed by calculating motility index (MI). The MI was defined as MI = \( \log_e (\text{sum of amplitudes} \times \text{total number of contraction waves} + 1) \) that is equivalent with the area under the contractility recording curve. The MI was calculated using a computer-assisted system (PowerLab, Chart version 4.2; AD-Instruments).

Duration of the stimulatory or inhibitory effects of acupuncture on gastric motility was measured between the time acupuncture was performed and when basal contractions returned after the acupuncture.

Results were expressed as means ± SE. Statistical analysis was performed by Student’s t-test (paired or unpaired 2 tail where appropriate). \( P < 0.05 \) was considered to be significant.

Materials. Rats were obtained from Charles River Laboratories (Raleigh, NC). Acupuncture needles were obtained from OMS Medical Supplies (Braintree, MA). Atropine, hexamethonium, atropine methyl bromide, and naloxone were obtained from Sigma Chemical (St. Louis, MO). These drugs were freshly prepared by dissolving in 0.2 ml of saline in each experiment.

RESULTS

Fasting gastric motor pattern in conscious rats. Two types of fasting gastric motor patterns were observed in the conscious condition of 35 rats tested (Fig. 1A). One type showed no cyclic groupings of strong contractions (type A), whereas the other (type B) showed cyclic groupings of strong contractions (phase III-like contractions) that are similar to the phase III contractions of the gastric MMC commonly observed in humans and dogs. Of 35 rats studied, 20 (57.1%) rats showed the type A pattern and 15 (42.9%) rats showed the type B pattern (Fig. 1A).

In type A rats, the peak amplitude and frequency of contractions were 5.18 ± 0.34 g and 3.93 ± 0.50 contractions/min (n = 20), respectively. In type B rats, the peak amplitude and frequency of each phase III-like contraction was 5.37 ± 0.36 g and 3.89 ± 0.43 contractions/min (n = 15), respectively. The duration and interval of the phase III-like contractions in type B rats were 5.4 ± 0.4 min and 9.5 ± 0.9 min (n = 15), respectively. During the experiments, both types of rats showed the same fasting gastric motor pattern (Fig. 2). Without acupuncture, type A rats never showed the cyclic groupings in the fasting gastric motor activity during the series of experiments.

Effects of acupuncture on gastric motility in type A rats. In 14 of 20 (70%) type A rats, acupuncture at ST-36 changed the fasting gastric motor pattern to that of type B (Fig. 1B). In addition, it significantly in-
creased the peak amplitude of contractions from $5.17 \pm 0.33$ to $10.52 \pm 0.41$ g/min (206.6 ± 22.1% of basal peak amplitude, $P < 0.05$, $n = 14$), inducing the strong phase III-like contractions in this group (Fig. 1B). The stimulatory effects of acupuncture were sustained for $3.52 \pm 0.21$ h after the 30 s of manual acupuncture (Fig. 3A). On the other hand, the acupuncture also suppressed the basal motor activity between the strong phase III-like contractions, inducing the phase I-like contractions as well (Figs. 1B and 3A). The MI for 60 min in this group was not significantly affected between before and after the acupuncture (before, 52.4 ± 3.9 vs. after, 39.2 ± 14.8 g/min, $n = 14$). However, when the MI after the acupuncture was calculated with the sum (60 min) of the duration of only phase I-like contractions, it was significantly decreased compared with the MI of basal contractions before the acupuncture ($52.4 \pm 3.9$ vs. $15.6 \pm 4.9$ g/min, $P < 0.05$, $n = 14$).

On the other hand, the other six (30%) type A rats showed no motor responses to acupuncture (Fig. 1B). The peak amplitude and MI ($5.19 \pm 0.35$ g and $55.9 \pm 5.0$ g/min, respectively) were not significantly affected after the acupuncture ($5.21 \pm 0.33$ g and $56.3 \pm 6.1$ g/min, respectively, $n = 6$).

In 3 of 14 type A rats that showed strong phase III-like contractions in response to acupuncture (Fig. 3A), the reproducibility was examined by repeating the

![Fig. 2. Fasting gastric motor pattern on separate days. Fasting gastric motor activity was recorded twice, on day 7 and day 10 after the surgery, in 3 type A (A) and type B rats (B). Fasting gastric motor pattern was not changed on day 10 compared with that on day 7. Representative data obtained from 1 rat in each type are shown.](http://ajpregu.physiology.org/)

![Fig. 3. Reproducible effects of acupuncture on gastric motility. In both type A and type B rats, acupuncture at stomach (ST-36 (arrowhead) was performed on day 7 (A, B) after the surgery. One week later (day 14), acupuncture at ST-36 was performed again in the same 3 rats of each group. On day 14, the stimulatory effects (*) and inhibitory effects (solid bars) of acupuncture in each rat were reproducible. Acupuncture at ST-36 also induced the phase I-like contractions (open bars) in type A rats (A). Representative data obtained from 1 rat in each type are shown.](http://ajpregu.physiology.org/)
same acupuncture 7 days later (on day 14 after surgery) to the same rat. In all three rats, acupuncture at ST-36 again converted the fasting motor pattern into that of the type B pattern (Fig. 3B). The peak amplitude after the acupuncture was not significantly affected between day 7 (10.56 ± 0.36 g) and day 14 (10.41 ± 0.35 g, n = 3). The MI after the acupuncture was also not significantly affected between day 7 (40.6 ± 14.4 g/min) and day 14 (40.8 ± 15.1 g/min, n = 3).

Overall, in type A rats (20 rats) acupuncture at ST-36 significantly increased the peak amplitude from 5.18 ± 0.34 to 9.01 ± 2.16 g (172.4 ± 25.6% of basal, P < 0.05, n = 20; Fig. 4A). In contrast, the MI was not affected between before (56.2 ± 5.2 g/min) and after the acupuncture (47.4 ± 12.3 g/min, 87.2 ± 20.2% of basal, n = 20; Fig. 4B).

Effects of acupuncture on gastric motility in type B rats. In 10 of 15 (66.6%) type B rats, acupuncture at ST-36 significantly decreased the peak amplitude of phase III-like contractions (Figs. 1C and 3C) from 5.39 ± 0.42 to 3.56 ± 0.43 g (66.3 ± 11.1% of basal, P < 0.05, n = 10). The inhibitory effects of acupuncture were sustained for 52.4 ± 10.3 min after 30 s of manual acupuncture. In addition, acupuncture at ST-36 significantly decreased the MI from 41.6 ± 2.1 to 30.1 ± 4.8 g/min (71.4 ± 11.8% of basal, P < 0.05, n = 10).

The other five (33.3%) type B rats showed no motor responses to the acupuncture (Fig. 1C). The peak amplitude and MI were not significantly affected between before (5.38 ± 0.20 g and 41.9 ± 1.6 g/min, respectively) and after the acupuncture (5.35 ± 0.26 g and 40.8 ± 1.5 g/min, respectively, n = 5). Among 15 type B rats studied, none showed an increase of the peak amplitude in response to acupuncture.

In 3 of 10 type B rats that showed suppressed phase III-like contractions in response to acupuncture (Fig. 3C), the reproducibility was examined 7 days later. In all three rats, acupuncture at ST-36 again suppressed the phase III-like contractions in a similar fashion (Fig. 3D). The peak amplitude after the acupuncture was not significantly affected between day 7 (5.39 ± 0.36 g) and day 14 (5.36 ± 0.41 g, n = 3). The MI after the acupuncture was also not significantly affected between day 7 (56.7 ± 2.2 g/min, n = 3) and day 14 (55.8 ± 2.4 g/min, n = 3).

Overall in type B rats (15 rats), acupuncture at ST-36 significantly decreased the peak amplitude from 5.37 ± 0.36 to 3.92 ± 0.76 g (72.9 ± 14.0% of basal, P < 0.05, n = 15; Fig. 4A) and the MI from 40.9 ± 3.2 to 30.1 ± 5.2 g/min (73.6 ± 16.2% of basal, P < 0.05, n = 15; Fig. 4B).

Effects of acupuncture at BL-21 on gastric motility in conscious rats. In both types of rats, acupuncture at BL-21 induced no significant effects on the fasting gastric motor pattern (Fig. 5). The peak amplitude and after the acupuncture at BL-21 were 5.29 ± 0.31 and 5.68 ± 0.91 g (103.4 ± 16.2% of basal, n = 20; Fig. 4A) in type A rats, respectively. The MI before and after the acupuncture at BL-21 were 55.3 ± 5.1 and 49.3 ± 7.8 g/min (90.5 ± 9.6% of basal, n = 20; Fig. 4B) in type A rats, respectively. The peak amplitude before and after the acupuncture at BL-21 was 5.41 ± 0.39 and 5.79 ± 1.20 g (105.0 ± 13.7% of basal, n = 15; Fig. 4A) in type B rats, respectively. The MI before and after...
the acupuncture at BL-21 was 41.6 ± 4.0 and 40.7 ± 3.7 g/min (98.5 ± 4.6% of basal, n = 15; Fig. 4B) in type B rats, respectively.

Effects of atropine, hexamethonium, and vagotomy on acupuncture-induced gastric contraction. To investigate whether a vagal-cholinergic pathway is involved in the stimulatory effects of acupuncture, either atropine or hexamethonium was administered 60 min after acupuncture. In type A rats, the strong phase III-like contractions induced by acupuncture were abolished by atropine (n = 4; Fig. 6A). Atropine significantly decreased the peak amplitude and MI for 60 min after the acupuncture from 10.62 ± 0.43 to 1.55 ± 0.11 g (14.2 ± 1.7% of control, P < 0.05, n = 4; Fig. 7A) and from 38.9 ± 14.0 to 7.2 ± 1.1 g/min (18.4 ± 3.9% of control, P < 0.05, n = 4; Fig. 7B), respectively. Similarly, the strong phase III-like contractions induced by acupuncture were abolished by hexamethonium (n = 3; Fig. 6B). Hexamethonium significantly decreased the peak amplitude and MI for 60 min after the acupuncture from 10.22 ± 0.61 to 1.64 ± 0.13 g (16.1 ± 1.9% of control, P < 0.05, n = 3; Fig. 7A) and from 39.9 ± 15.3 to 8.8 ± 1.3 g/min (19.1 ± 4.2% of control, P < 0.05, n = 3; Fig. 7B), respectively.

Atropine methyl bromide also abolished the strong phase III-like contractions induced by acupuncture (n = 3; Fig. 6C). Atropine methyl bromide significantly decreased the peak amplitude and MI for 60 min after the acupuncture from 10.89 ± 0.50 to 1.63 ± 0.14 g (15.1 ± 1.9% of control, P < 0.05, n = 3; Fig. 7A) and from 39.5 ± 14.7 to 7.9 ± 2.6 g/min (20.2 ± 4.0% of control, P < 0.05, n = 3; Fig. 7B), respectively.

In type A rats, vagotomy significantly decreased the peak amplitude and MI from 5.48 ± 0.32 to 2.08 ± 0.23 g (36.1 ± 8.8% of control) and from 55.8 ± 5.6 to 16.3 ± 0.13 g/min (29.0 ± 2.8% of control, P < 0.05, n = 4), respectively. In contrast, it significantly increased the frequency of contractions from 3.93 ± 0.42 to 5.45 ± 0.40 contractions/min (P < 0.05, n = 4).

Acupuncture failed to induce any stimulatory effects on the fasting gastric motility in vagotomized type A rats (Fig. 8). The peak amplitude and MI were not significantly affected between before (2.07 ± 0.12 g and 17.1 ± 0.10 g/min, respectively) and after the acupuncture (2.06 ± 0.11 g and 16.9 ± 0.11 g/min, respectively, n = 4).

In type B rats, the basal phase III-like contractions were abolished by the administration of atropine and hexamethonium. Atropine significantly decreased the peak amplitude and MI from 5.39 ± 0.29 to 1.62 ± 0.13 g and from 42.8 ± 3.1 to 8.9 ± 1.3 g/min (P < 0.05, n = 4), respectively. Similarly, hexamethonium significantly decreased the peak amplitude and MI from 5.40 ± 0.27 to 1.59 ± 0.11 g and from 41.9 ± 4.1 to 9.1 ± 1.3 g/min (P < 0.05, n = 3), respectively. These results suggest that the basal phase III-like contractions are mediated by a cholinergic pathway. Therefore, the effects of atropine, hexamethonium, and vagotomy on the inhibitory effects of acupuncture were not studied further in type B rats.

Effects of naloxone on acupuncture-induced gastric contraction. The stimulatory effects of manual acupuncture (30 s) on gastric motility were sustained for over 3 h in type A rats (mean duration time: 3.52 ± 0.21 h, n = 3; Fig. 9A). Naloxone (5 mg/kg ip) itself had no significant effect on the fasting gastric motor activity in type A rats. Basal peak amplitude and MI (5.17 ± 0.32 g and 56.4 ± 4.9 g/min, respectively) were not significantly affected by naloxone (5.16 ± 0.36 g and 56.6 ± 5.2 g/min, respectively, n = 3; Fig. 9B).

In contrast, pretreatment with naloxone significantly shortened the duration of the phase III-like contractions induced by acupuncture from 3.52 ± 0.21 to 1.02 ± 0.15 h (P < 0.05, n = 3; Fig. 9B). In addition, naloxone significantly decreased the peak amplitude and MI for 3 h after the acupuncture from 10.51 ± 0.92 to 3.9 ± 1.6 g and from 156.6 ± 19.7 to 59.9 ± 9.3 g/min (P < 0.05, n = 3), respectively.

DISCUSSION

It is widely accepted that the gastric MMC occurs in humans (38, 54) and dogs (19, 52) in the fasting state. It has been shown in rats that the MMC is observed in the duodenum (2, 43). It remains, however, controversial whether rats have a similar MMC in the stomach in the fasting condition. In several studies using myoelectrographic recordings (43) and mechanical recordings (15, 25, 51), no obvious gastric MMC was shown in rats. In contrast, recent study showed the gastric phase III-like contractions in rats (27). The results obtained from type A rats in this study, those showing no cyclic groupings of strong contractions, are consistent with the results of previous studies, showing no obvious gastric MMC in rats. On the other hand, our results showed that only 42.9% (type B) of the rats showed cyclic groupings of the phase III-like contractions. In addition, the same rat showed the same type of fasting gastric motor pattern during the experiments. Because the gastric contractions of the phase III are mediated by the activation of vagal efferent in dogs (19, 39, 52), the difference of vagal efferent activity could explain the difference of the fasting motor pattern observed between type A and type B rats. In general, the rat stomach never gets empty in any physiological conditions. Therefore, each rat could show different vagal activity once they are fasted for 24 h. The mechanism(s) underlying the different fasting motor pattern remains to be explored.

The effects of acupuncture on gastric motility are still controversial. Manual acupuncture at ST-36 inhibited antral motility in rabbits (23, 37). On the contrary, electroacupuncture at ST-36 stimulated antral motility in rabbits and cats (33, 36). In humans, it has been shown that electroacupuncture at ST-36 either increased or decreased gastric peristalsis, depending on the initial gastric activity (61). Similarly, Qian and Lin (46) showed the dual effects of electroacupuncture, either enhancing the hypofunction or reducing the hyperfunction of the pyloric peristalsis. It seems that the effects of (electro) acupuncture on gastric motility de-
Fig. 6. Effects of atropine (A), hexamethonium (B), and atropine methyl bromide (C) on acupuncture-induced gastric motility in type A rats. Strong phase III-like contractions (*) induced by acupuncture at ST-36 (arrowhead) were abolished by atropine (50 μg/kg sc), hexamethonium (20 mg/kg sc), and atropine methyl bromide (1 mg/kg sc). Results were reproducible in 3 or 4 different rats.

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pend on various factors such as species, acupuncture procedure, and initial basic motor activity, etc. According to Chinese traditional medicine, acupuncture is generally believed to balance the energy flows of Yin and Yang, the two major “negative” and “positive” forces governing the body (10, 34, 57). It is also believed that acupuncture exerts different effects on internal organs to restore the homeostatic balance. No scientific evidence so far clarifies the mechanism(s) contributing the dual effects of (electro) acupuncture.

In this study, acupuncture at ST-36 significantly decreased the peak amplitude of the phase III-like contractions and MI in type B rats. In contrast, it significantly increased the peak amplitude of contractions but not the MI in type A rats. This is due to the fact that the acupuncture in this group also suppressed the basal motor activity between the strong phase III-like contractions. As a result, it induced the phase I-like contractions as well as the phase III-like contractions, changing the motor pattern to the obvious phase I- and phase III-like cycles usually observed in humans and dogs (19, 39, 52). These results suggest that acupuncture induces stimulatory and/or inhibitory effects on gastric motility, not only in the different types of rats but also in each rat. The cyclic gastric MMC (phase I-IV) are regulated under the vagal activity in humans and dogs (19, 39, 52). The vagal activity in type A rats may be monotonous whereas that in type B rats may be varied (cyclic) in fasting condition. Thus the effects of acupuncture on gastric motility may depend on the basal vagal activity. This remains to be explored.

The nucleus of the solitary tract (NTS) at the brain stem receives visceral sensory inputs as well as somatosensory inputs (55). In our previous study, somato-autonomic reflexes mediate the gastric relaxations in response to acupuncture at the abdominal wall. Acupuncture significantly increased the number of c-fos-immunopositive neurons in the NTS and the rostral ventrolateral medulla (RVLM), but not in the dorsal motor nucleus vagi (DMV) (49), suggesting neural activation in the NTS and the RVLM. The RVLM sends the inhibitory outputs to the stomach via sympathetic efferents. In contrast, it has been shown that food in the stomach induces neural activation in the NTS and the DMV (13). These findings indicate that the NTS is involved, at least in part, in pathways to both the inhibitory (sympathetic) and the excitatory (parasympathetic) neural outputs to the stomach. Therefore, somatosensory inputs into the NTS might modulate other neural circuitry in the NTS positively or negatively, thus projecting either stimulatory or inhibitory outputs to the stomach despite the same neural inputs into the NTS. It is conceivable that once the parasympathetic nerve is activated, acupuncture may enhance the sympathetic activity to overcome the parasympathetic activity, resulting in suppressing the MMC. On the other hand, once the sympathetic nerve is activated, acupuncture may enhance the parasympathetic activity to overcome the sympathetic activity, resulting in enhancing the MMC.

We further studied the mechanism of the stimulatory effects of acupuncture on gastric motility in type A rats. The stimulatory effects of acupuncture were abolished by atropine and hexamethonium. Atropine methyl bromide also abolished the phase III-like contractions induced by acupuncture, suggesting that acupuncture-induced phase III-like contractions are mediated via peripheral muscarinic receptors. Furthermore, the stimulatory effects of acupuncture were not observed in vagotomized rats. On the other hand, it has been shown that the vagal outputs are modulated by the sympathetic inputs via $\alpha_2$-adrenoreceptors (12, 20).

![Vagotomized rat](http://ajpregu.physiology.org/)

**Fig. 8.** Effects of truncal vagotomy on acupuncture-induced gastric motility in type A rats. In vagotomized type A rats, acupuncture at ST-36 (arrowhead) had no effects on the gastric motility. Results were reproducible in 4 different rats.

**Fig. 7.** Peak amplitude (A) and motility index (B) after acupuncture in type A rats. Peak amplitude after the acupuncture, but before drug administration, was set as 100% and compared between before and after drug administration (A). Motility index for 60 min before (but after the acupuncture) and after drug administration were compared (B). *P < 0.05.
Thus it still remains unknown whether stimulating the vagal pathway or inhibiting the sympathetic pathway mediates the stimulatory effects of acupuncture on gastric motility. The results in this study suggest that the stimulatory effects of acupuncture on gastric motility are mediated, at least in part, via cholinergic vagal pathways.

In urethane-anesthetized rats, Sato et al. (48) demonstrated that the acupuncture at ST-36 simulated gastric motility via a somato-parasympathetic reflex. However, our present study demonstrated that the stimulatory effects of acupuncture observed in conscious rats was sustained for over 3 h after the 30 s of manual acupuncture. This indicates that simple neural reflexes are not the only pathways in mediating the stimulatory effects of acupuncture on gastric motility.

The inhibitory effects of electroacupuncture on gastric acid output (56) and sympathetic cardiovascular response (6, 31, 32) are both antagonized by naloxone. It has been shown that electroacupuncture at ST-36 induces the expression of opioid peptides and receptors in the central nervous system (14, 18). Several studies of acupuncture with manual (40, 48) and electric stimulation (58) have shown the transient and long-lasting effects of acupuncture. It has been shown that the stimulation of manual acupuncture with low frequency induction leads to long-lasting effects via an opioid-sensitive pathway, whereas that with high-frequency electric stimulation induces short-lasting effects via an opioid-insensitive pathway (29, 44). The stimulation of manual acupuncture used in this study was supposed to be low frequency (~1 Hz), because the needle was twisted once every second. Therefore, it is conceivable that the induction of opioids and/or opioid receptor(s) gene expression may mediate the long-lasting effects of manual acupuncture.

We showed that the long-lasting stimulatory effects of manual acupuncture on gastric motility were attenuated by naloxone given peripherally. This suggests that the stimulatory effects of acupuncture on gastric motility are mediated via opioids as well. However, it still remains unclear whether the stimulatory effects of acupuncture on gastric motility are mediated via centrally released opioids or peripherally released opioids. It has been shown that the effects of central opioids on gastric motility are inhibitory (3). In vitro study has suggested that within the DMV, the µ-opioid receptor is involved in inhibiting the excitatory, but not inhibitory, synaptic transmissions (4). It has been shown that peripheral administration of morphine induces gastric phase III-like activity (30). Therefore, one possibility is that peripheral opioids could mediate the long-lasting stimulatory effects of acupuncture on gastric motility in conscious rats.

On the other hand, recent studies showed that opioid receptors (especially µ- and δ-receptors) in the RVLM mediate the inhibitory effects of electroacupuncture (6, 31, 32). It has also been shown that electroacupuncture at ST-36 leads the induction of the central opioid system, both in peptide and receptor levels (14, 18). There-
fore, we cannot exclude the possibility that the stimulatory effects of acupuncture on gastric motility are mediated via central opioids outside the DMV. Further studies exploring region-specific roles of central μ-, δ-, and κ-opioid receptors on gastric motility will provide new insights into the mechanism(s) underlying the acupuncture-induced gastric motility.

The effects of acupuncture on GI function have only recently been investigated with electroacupuncture (5, 41, 45, 46, 56). However, during over 5,000 years history of traditional Chinese medicine, acupuncture has been used without electric stimulation. In the present study, gastric motility was not significantly affected by acupuncture in ~30% of each type of rats. It seems that no response to acupuncture observed in some of type A and type B rats (nonresponders) could be due to the short duration (30 s) of manual acupuncture. We have, therefore, performed electroacupuncture (2–5 V, 0.7-ms pulse duration, 1–3 mA, 1 Hz, for 30 min) at ST-36 in rats that have not responded to manual acupuncture. Neither stimulatory nor inhibitory responses were, however, observed in response to electroacupuncture in four rats of type A and four rats of type B of nonresponders to manual acupuncture (unpublished observations). Thus it is suggested that no response to acupuncture observed in some of the type A and type B rats is not due to the short duration of manual acupuncture. We do believe that manual manipulation of acupuncture needle is still a common method for the current complementary alternative medicine. We also believe that manual manipulation affects GI motility effectively. In fact, our recent study showed that 60-s manual acupuncture on the lower abdomen caused a transient gastric relaxation in anesthetized rats (49). A similar observation was reported by other investigators (48).

In summary, manual acupuncture at ST-36 on the hindlimb induces dual effects on gastric motility in conscious rats. The stimulatory effects were observed only in rats that showed no gastric MMC pattern. The inhibitory effects were mainly observed in rats that showed gastric MMC pattern. The stimulatory effects of acupuncture are mediated via vagal efferent and opioid pathways. Modulation of opioid pathway(s) by acupuncture may contribute to the long-lasting effects of acupuncture on gastric motility in conscious rats.

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