

Acupuncture at heterotopic acupoints enhances jejunal motility in constipated and diarrheic rats

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Abstract

AIM: To investigate the effect and mechanism of acupuncture at heterotopic acupoints on jejunal motility, particularly in pathological conditions.

METHODS: Jejunal motility was assessed using a manometric balloon placed in the jejunum approximately 18-20 cm downstream from the pylorus and filled with approximately 0.1 mL warm water in anesthetized normal rats or rats with diarrhea or constipation. The heterotopic

acupoints including LI11 (Quchi), ST37 (Shangjuxu), BL25 (Dachangshu), and the homotopic acupoint ST25 (Tianshu), were stimulated for 60 s by rotating acupuncture needles right and left at a frequency of 2 Hz. To determine the type of afferent fibers mediating the regulation of jejunal motility by manual acupuncture, the ipsilateral sciatic A or C fibers of ST37 were inactivated by local application of the A-fiber selective demyelination agent cobra venom or the C fiber blocker capsaicin. Methocitramine, a selective M₂ receptor antagonist, was injected intravenously to identify a specific role of M₂ receptors in mediating the effect of acupuncture on jejunal motility.

RESULTS: Acupuncture at heterotopic acupoints, such as LI11 and ST37, increased jejunal motility not only in normal rats, but also in rats with constipation or diarrhea. In normal rats, manual acupuncture at LI11 or ST37 enhanced jejunal pressure from 7.34 ± 0.19 cmH₂O to 7.93 ± 0.20 cmH₂O, an increase of $9.05\% \pm 0.82\%$ ($P < 0.05$), and from 6.95 ± 0.14 cmH₂O to 8.97 ± 0.22 cmH₂O, a significant increase of $27.44\% \pm 1.96\%$ ($P < 0.01$), respectively. In constipated rats, manual acupuncture at LI11 or ST37 increased intrajejunal pressure from 8.17 ± 0.31 cmH₂O to 9.86 ± 0.36 cmH₂O, an increase of $20.69\% \pm 2.10\%$ ($P < 0.05$), and from 8.82 ± 0.28 cmH₂O to 10.83 ± 0.28 cmH₂O, an increase of $22.81\% \pm 1.46\%$ ($P < 0.05$), respectively. In rats with diarrhea, MA at LI11 or ST37 increased intrajejunal pressure from 11.95 ± 0.35 cmH₂O to 13.96 ± 0.39 cmH₂O, an increase of $16.82\% \pm 2.35\%$ ($P < 0.05$), and tended to increase intrajejunal pressure (from 12.42 ± 0.38 cmH₂O to 13.05 ± 0.38 cmH₂O, an increase of $5.07\% \pm 1.08\%$, $P > 0.05$), respectively. In contrast, acupuncture ST25, a homotopic acupoint, not only decreased intrajejunal pressure, but also significantly decreased frequency in normal rats and rats with constipation or diarrhea. Following demyelination of A δ fibers, acupuncture at ST37 again augmented intrajejunal pressure to $121.48\% \pm 3.06\%$ of baseline. Following capsaicin application for 24 h, acupuncture at ipsilateral ST37 increased intrajeju-

nal pressure to $106.63\% \pm 1.26\%$ of basal levels when compared to measurements prior to capsaicin treatment ($P < 0.05$). Acupuncture at LI11, ST37, or BL25 significantly rescued methoctramine-mediated inhibition of jejunal motility amplitude from $42.83\% \pm 1.65\%$ to $53.43\% \pm 1.95\%$ of baseline ($P < 0.05$), from $45.15\% \pm 2.22\%$ to $70.51\% \pm 2.34\%$ of baseline ($P < 0.01$), and from $38.03\% \pm 2.34\%$ to $70.12\% \pm 2.22\%$ of baseline ($P < 0.01$), respectively.

CONCLUSION: Acupuncture at heterotopic acupoints increases the amplitude of jejunal motility in rats. C fibers and M₂ receptors predominantly and (or) partially mediate the regulation of jejunal motility by acupuncture, respectively.

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Key words: Acupuncture; Heterotopic acupoint; LI11; ST37; BL25; Jejunal motility; Constipation; Diarrhea; C fibers; Muscarinic receptors

Core tip: This is the first study we know of that investigates the effect and mechanism of acupuncture at heterotopic acupoints on jejunal motility in normal rats or rats with constipation or diarrhea. We observed that acupuncture at heterotopic LI11 or ST37 points increased jejunal motility regardless of initial condition. We demonstrated that acupuncture applied at these points regulated jejunal motility by activating A δ and C afferent fibers; however, the latter predominates. M₂ receptors play a role in this process.

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INTRODUCTION

Gastrointestinal motility disorders contribute to many diseases, including impaired accommodation, gastroparesis, dumping syndrome, constipation, and diarrhea. Many studies have been conducted to explore the efficacy of somatic stimulation for the treatment of gastrointestinal motility disorders^[1-5]. Reproducible results have been generated in both clinical and research settings^[6,7]. It has been repeatedly shown that the effect of acupuncture on gastric motility is primarily associated with autonomic reflexes and the gut-brain axis^[8]. Stimulation at homotopic acupoints, where afferent innervation is in the same segment from which the efferents innervate visceral organs, decreases intragastric pressure with or without spinalization, and acupuncture at heterotopic acupoints, involving different segmental innervation of the spinal cord to visceral organs, induces gastrointestinal facilitation only

in complete spinal rats^[9]. However, previous studies have rarely stated the effects of acupuncture on gastrointestinal motility in pathological conditions, and little effort has been made to investigate the effects of acupuncture on small intestinal motility. Furthermore, the precise mechanism of acupuncture on small intestinal motility requires clarification.

Acupuncture treatment involves the insertion of thin needles into the skin and underlying muscle layer. Previous studies have demonstrated that gastrointestinal motility is influenced by somatic afferent stimulation^[10-14]. Sensory stimulation of the abdominal skin by pinching inhibits gastric motility, whereas similar stimulation of a hind paw enhances gastric motility in rats^[15]. We have previously provided evidence that only stimulation intensity above a threshold for activating A δ and/or C fibers can modulate gastric motility^[9].

Parasympathetic nerves play a critical role in the excitatory regulation of gastrointestinal motility by acupuncture or acupuncture-like stimulation at heterotopic acupoints, such as ST36 (Zusanli), ST37 (Shangjuxu), and LI11 (Quchi)^[9]. The majority of postganglionic fibers derived from parasympathetic nerves show a cholinergic component that releases acetylcholine (ACH) in the terminals when activated. ACH then activates smooth muscle cell surface muscarinic receptors directly or indirectly, thus triggering various intracellular signaling pathways, which lead to a rise in cytosolic Ca²⁺ concentration and, eventually, smooth muscle contraction^[16-18].

Here, we examined the effect of acupuncture at heterotopic acupoints on jejunal motility and tested the hypothesis that activation of A δ and/or C fibers may be required for the regulation of jejunal motility by acupuncture at heterotopic acupoints and that M₂/M₃ receptors mediate this effect.

MATERIALS AND METHODS

Animal preparation

Animal experiments were performed in accordance with the National Institutes of Health's Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of the China Academy of Chinese Medical Sciences. Adult male Sprague-Dawley rats (SD) weighing 250-300 g were housed with free access to food and water. The animals were maintained on a light-dark cycle (dark cycle 8:00 pm-8:00 am) and acclimated for seven days prior to the experiment. Acute diarrhea was induced in rats by administering a senna solution containing 0.3 g/mL crude drug liquid *via* oral gavage at a dose of 10 mL/kg per day for two consecutive days^[19]. Constipated rats were generated *via* oral gavage of 0 °C normal saline, 10 mL/kg per day for five consecutive days^[20].

Recording of jejunal motility

Rats were fasted overnight prior to surgery and electrophysiological recordings. They were initially anesthetized

with intraperitoneal injections of urethane (1.0–1.2 g/kg, *ip*), with supplementary anesthesia (0.1 g/kg *ip*) being administered if withdrawal of limbs or a fluctuation of the heart rate was observed. Core body temperature was monitored and maintained at $37.0 \pm 0.5^\circ\text{C}$ by a feedback controlled electric blanket (ALC-HTP, Shanghai Alcott Biotech Co., Ltd, China). A median abdominal incision was generated and one manometric balloon was placed into the jejunum at approximately 18–20 cm downstream from the pylorus. The balloon was filled with approximately 0.1 mL warm water and connected to a piece of polyethylene tube, providing pressure of approximately 100 mm H₂O. Pressure in the intestinal lumen was measured with a transducer through the polyethylene tube and recorded using a Mac Lab system (AD Instruments, Australia). Heart rate was also monitored to maintain anesthetic depth and to avoid marked cardiac fluctuations caused by drug administration. At 30 min after stable recording of jejunal motility, acupuncture stimulation was conducted randomly on different acupoints.

Acupuncture stimulation

In this study, the heterotopic acupoints included LI11 (Quchi), ST37 (Shangjuxu), and BL25 (Dachangshu), with the homotopic acupoint ST25 selected as a control. LI11 locates to the midpoint between the lateral end of the transverse cubical crease and the lateral epicondyle of the humerus; ST25 is level with the navel, 2 mm lateral to anterior median line; ST37 is 5 mm lateral to the anterior tubercle of the tibia and 15 mm below the knee joint; BL25, at the waist, is under the fourth lumbar spine and 5 mm lateral to posterior midline. The acupuncture needles (0.25 mm \times 25 mm, Suzhou Hwato Medical Instruments, China) were inserted unilaterally, vertically, or slightly obliquely, to a depth of approximately 5 mm and rotated right then left, at a frequency of 2 Hz for 60 s.

Afferent fiber inactivation

Ipsilateral sciatic A or C fibers of ST37 were inactivated. The sciatic nerve A fibers were demyelinated using cobra venom as described previously^[21]. In brief, the sciatic nerve trunk was isolated 1 cm over the fibular capitulum in anesthetized rats. A Hamilton syringe was used to aspirate 1 μL normal saline, 1 μL air, and 1 μL 0.3% cobra venom solution in sequence, the needle tip was then inserted into the sciatic nerve membrane and the above solution was injected into the myelin sheath while avoiding drug leakage into the surrounding tissue. Acupuncture stimulation was repeated at 30 min after venom injection and suture of the incision.

The unilateral sciatic nerve C fibers were inactivated by capsaicin in anesthetized rats^[22]. Briefly, the sciatic nerve was isolated 1 cm above the fibular capitulum, and the nerve trunk was wrapped with gauze immersed in 2% capsaicin solution for 24 h. The incision was sutured aseptically for healing. At 24 h, acupuncture was performed.

Drug administration

Methocitramine (0.5 mg/kg, Sigma, United States), a se-

lective M₂ cholinergic receptor antagonist, was used in this study and diluted to 0.05% with normal saline. The diluted antagonist was injected intravenously within 1–2 min to avoid marked fluctuation of the heart rate. Acupuncture commenced when the effect of injection of the compound on heart rate and jejunal motility recovered.

Statistical analysis

All data were analyzed online and offline using the Mac Lab system. Spontaneous intestinal peristalses were recorded continuously prior to and during acupuncture stimulation. For this study, the frequency and amplitude of intestinal motility was analyzed. Statistical analysis was performed using SPSS17.0 software (IBM, United States). All data were shown as the mean \pm SE. To evaluate statistical significance, data sets with normal distribution were analyzed using paired or unpaired *t*-test in the case of two groups or using one-way ANOVAs followed by *q* tests in the case of more than two groups. *P* < 0.05 was considered statistically significant.

RESULTS

Acupuncture at heterotopic acupoints induces an excitatory effect on jejunal motility in normal rats

Baseline of jejunal motility was recorded using the water-filled balloon, and intrajejunal pressure was stabilized at a baseline of approximately 100 cmH₂O. Peristalsis waves comprised high frequency (about 30 times/min) and low amplitude (about 8 cmH₂O) circular muscle contractions, and transitory waves comprised low frequency (about 8 times/min) and high amplitude (about 20 cmH₂O) longitudinal muscle contractions; both were obtained by the inbuilt balloon pressure detection system. The peristalsis waves showed more stable amplitude and duration than the transitory waves, and the average frequency and amplitude of jejunal peristalsis waves were 29.86 ± 1.52 times/min and 7.45 ± 0.65 cmH₂O, respectively, in normal rats (Figure 1A). Therefore, the peristalsis waves were used to analyze the effect of acupuncture in this study.

In order to determine whether acupuncture at heterotopic acupoints affects jejunal motility in normal rats, we designed an experiment in which manual acupuncture (MA) was applied separately at LI11, ST37, ST25, and BL25. As shown in Figures 1B, C, D, and E, MA at LI11 increased jejunal pressure from 7.34 ± 0.19 cmH₂O to 7.93 ± 0.20 cmH₂O, a significant increase of $9.05\% \pm 0.82\%$ (*P* < 0.05, *n* = 17). MA at ST37 enhanced jejunal pressure from 6.95 ± 0.14 cmH₂O to 8.97 ± 0.22 cmH₂O, which was also significant ($27.44\% \pm 1.96\%$, *P* < 0.01). However, the above acupoints did not significantly change the jejunal motility frequency (MA at LI11: from 29.94 ± 0.39 times/min to 29.65 ± 0.32 times/min, *P* > 0.05, *n* = 17; MA at ST37: from 29.65 ± 0.34 times/min to 28.82 ± 0.33 times/min, *P* > 0.05, *n* = 17). MA at ST25 decreased intrajejunal pressure from 8.12 ± 0.14 cmH₂O to 5.3 ± 0.14 cmH₂O, a significant effect ($32.56\% \pm 1.49\%$, *P* < 0.01, *n* = 17), and frequency from 30.75 ± 0.37 times/min to

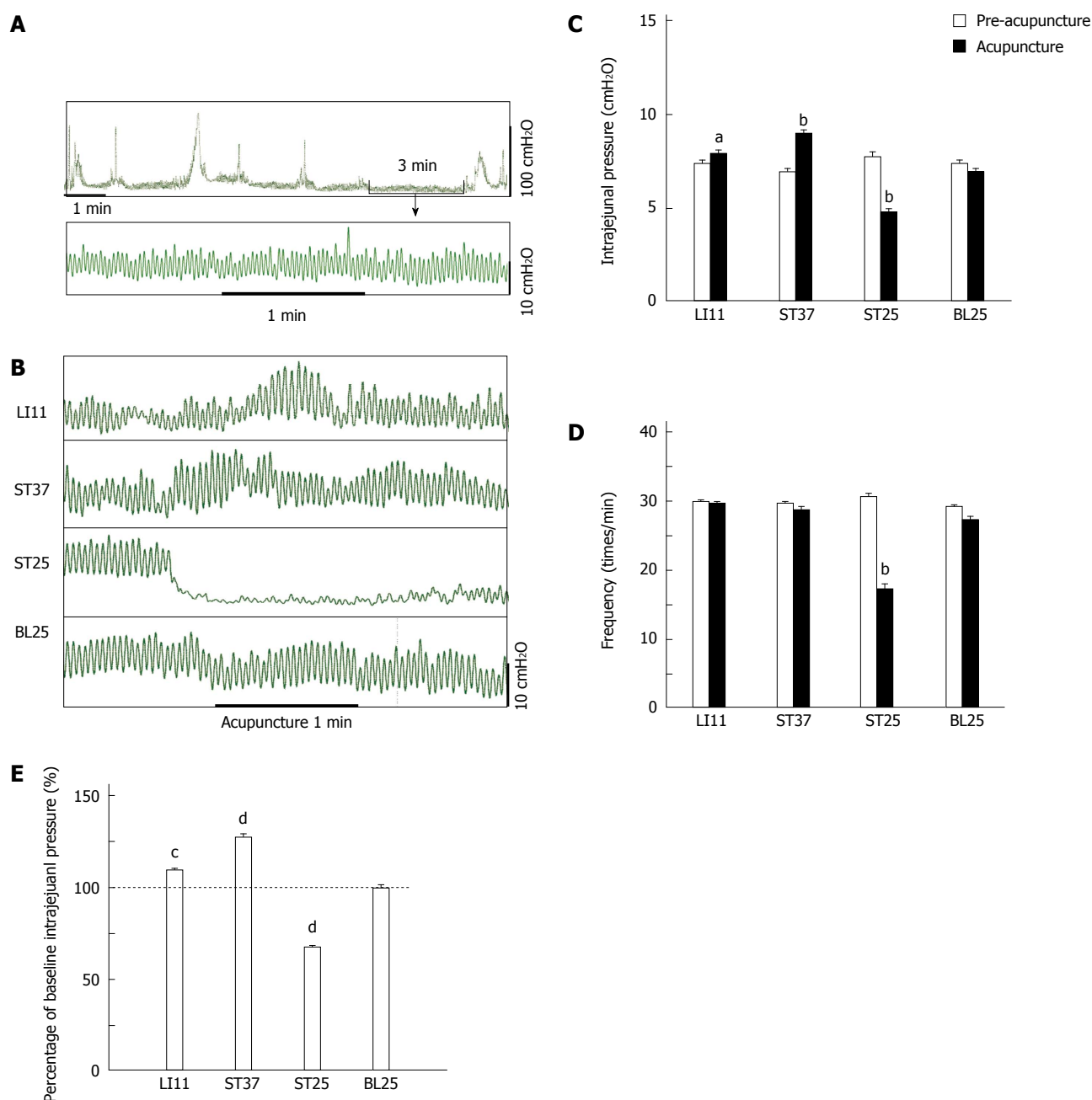


Figure 1 Jejunal motility regulated by manual acupuncture at LI11, ST37, ST25, and BL25 in normal rats. A: A representative trace of jejunal motility in normal rat; B: Representative traces of jejunal motility regulated by acupuncture at LI11, ST37, ST25, and BL25; C: Acupuncture at LI11, ST37, and ST25 affected intrajejunal pressure significantly in normal rats (^a $P < 0.05$, ^b $P < 0.01$ vs pre-acupuncture, paired t -test, $n = 17$); D: Only acupuncture at ST25 decreased the frequency of jejunal motility (^b $P < 0.01$ vs pre-acupuncture, paired t -test, $n = 17$); E: The percent change in intrajejunal pressure caused by acupuncture at LI11, ST37, ST25, and BL25 (^c $P < 0.05$, ^d $P < 0.01$ vs baseline, paired t -test, $n = 17$). Intrajejunal pressure was normalized by baseline, which is denoted by the dashed line.

17.31 ± 0.74 times/min, also a significant effect ($46.99\% \pm 1.98\%$, $P < 0.01$, $n = 17$). MA at BL25 did not change intrajejunal pressure (from 7.42 ± 0.16 cmH₂O to 6.98 ± 0.15 cmH₂O, $P > 0.05$, $n = 17$) or motility frequency (from 29.18 ± 0.41 times/min to 27.29 ± 0.45 times/min, $P > 0.05$, $n = 17$) significantly. The above results suggest that in normal rats, MA at the heterotopic acupoints LI11 and ST37 may increase jejunal motility, with an opposite effect of that observed in MA at the homotopic acupoints ST25.

Acupuncture at heterotopic acupoints enhances jejunal motility in rats with constipation

Previous studies have reported the dual effects of acupuncture at heterotopic acupoints on gastric motility^[23-26]. For example, Tatewaki *et al*^[24] showed that manual acupuncture at ST36 induced dual effects: stimulating gastric contractions in rats with hypomotility and inhibiting gastric contractions in rats with hypermotility. This raised the question as to whether the effect of acupuncture at heterotopic acupoints on jejunal motility could be re-

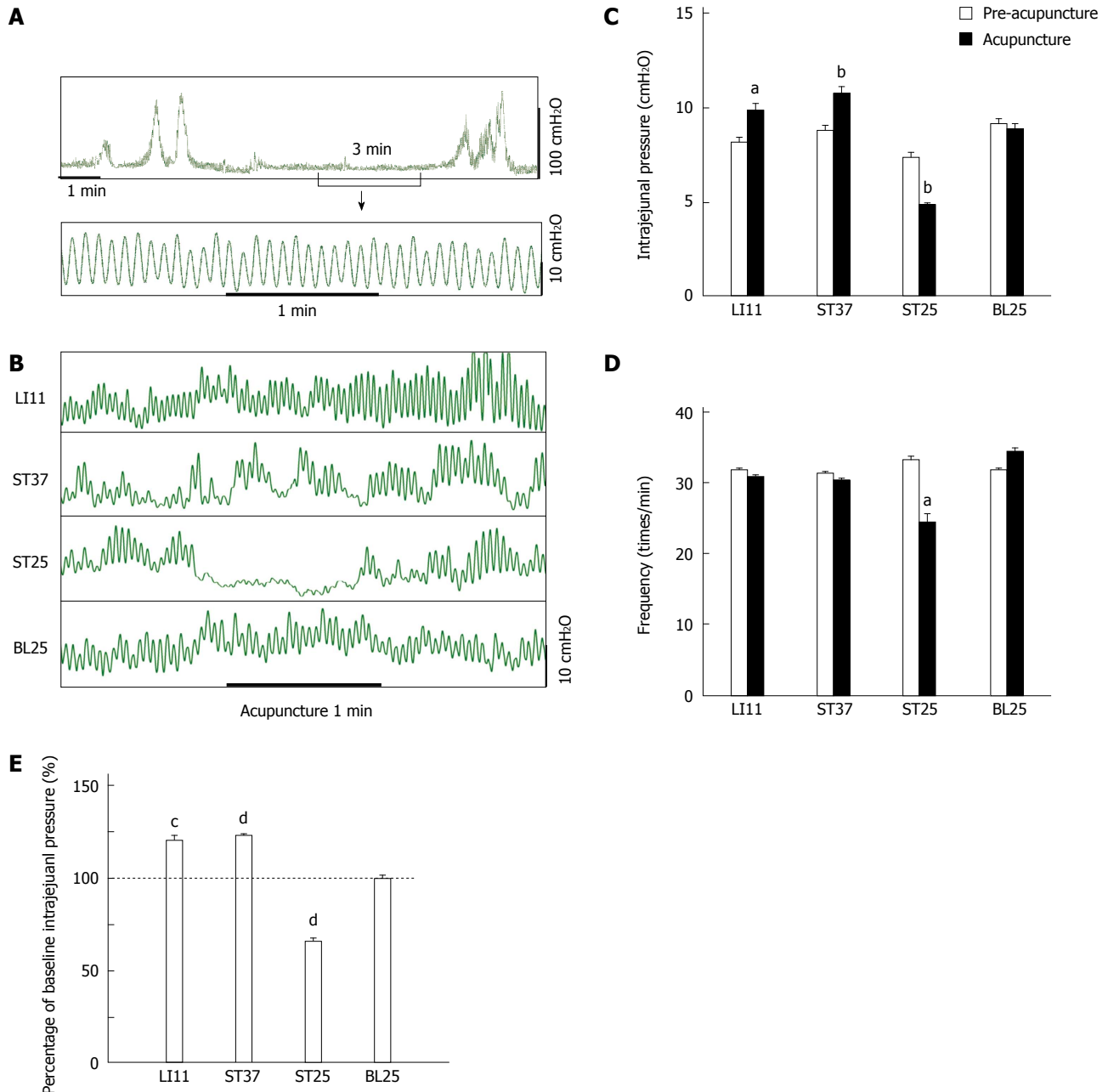


Figure 2 Effects of manual acupuncture at LI11, ST37, ST25, and BL25 on jejunal motility in constipated rats. A: A representative trace of jejunal motility in a constipated rat; B: Representative traces of jejunal motility regulated by acupuncture at LI11, ST37, ST25, and BL25 in constipated rats; C: Intrajejunal pressure was enhanced by acupuncture at LI11 or ST37 but reduced by acupuncture at ST25 (^a $P < 0.05$, ^b $P < 0.01$ vs pre-acupuncture, paired t -test, $n = 17$); D: Only acupuncture at ST25 decreased the frequency of jejunal motility (^a $P < 0.05$ vs pre-acupuncture, paired t -test, $n = 17$); E: The percent change in intrajejunal pressure caused by acupuncture at LI11, ST37, ST25, and BL25 (^c $P < 0.05$, ^d $P < 0.01$ vs baseline, paired t -test, $n = 17$). The intrajejunal pressure was normalized by baseline, which is denoted by the dashed line.

produced in rats with functional intestinal diseases. To answer this, we generated constipated rats with intestinal hypomotility and rats with diarrhea with hypermotility to identify whether MA at the above acupoints could induce effects on jejunal motility similar to those noted in normal rats.

Firstly, the irritable model of constipation in rats was generated using a five-day intragastric infusion of 0 °C ice normal saline (10 mL/kg). At 3 h after infusion on the first two days, a higher defecation frequency was

observed and the excrement changed to soft and loose. Over the remaining part of the day, defecation of hard stool was observed at a lower frequency. The rats produced fewer and harder stools on days 3 to 5. The stool moisture reduced from $40.23\% \pm 2.19\%$ to $29.37\% \pm 4.65\%$ ($P < 0.05$, $n = 17$) following five days of ice-water infusion, indicating the model had developed fully. In constipated rats, the baseline amplitude and frequency of jejunal motility waves were as follows: for peristalsis waves the frequency was 32.52 ± 0.18 times/min and the

amplitude 8.43 ± 0.14 cmH₂O; for translatory waves the frequency was 5–6 times/min and the amplitude about 20 cmH₂O (Figure 2A).

As shown in Figures 2B, C, and D, MA at LI11 increased intrajejunal pressure from 8.17 ± 0.31 cmH₂O to 9.86 ± 0.36 cmH₂O, a significant increase of $20.69\% \pm 2.10\%$ ($P < 0.05$, $n = 17$); MA at ST37 enhanced intrajejunal pressure from 8.82 ± 0.28 cmH₂O to 10.83 ± 0.28 cmH₂O with a significant increase of $22.81\% \pm 1.46\%$ ($P < 0.05$, $n = 17$). However, frequency was not changed significantly by MA at the above two acupoints (MA at LI11: from 32.12 ± 0.29 times/min to 31.23 ± 0.23 times/min, $P > 0.05$, $n = 17$; MA at ST37: from 31.57 ± 0.26 times/min to 30.71 ± 0.37 times/min, $P > 0.05$, $n = 17$). Interestingly, MA at ST25 significantly decreased not only intrajejunal pressure from 7.73 ± 0.24 cmH₂O to 4.82 ± 0.14 cmH₂O (a decrease of $34.39\% \pm 1.66\%$, $P < 0.01$, $n = 17$), but also frequency from 33.63 ± 0.43 times/min to 24.78 ± 1.12 times/min ($P < 0.05$, $n = 17$), a decrease of $22.37\% \pm 2.52\%$. Similar to results in normal rats, MA at BL25 did not change intrajejunal pressure or frequency of jejunal motility markedly (intrajejunal pressure: 9.20 ± 0.27 cmH₂O for baseline, 8.95 ± 0.24 cmH₂O for MA, $P > 0.05$; frequency: 32.04 ± 0.32 times/min for baseline, 34.77 ± 0.38 times/min for MA, $P > 0.05$, $n = 17$). These results suggest that in constipated rats, acupuncture at LI11 and ST37 may increase jejunal motility and that MA at ST25 nevertheless showed an inhibitory effect on motility.

Acupuncture at heterotopic acupoints increases jejunal motility in a rat model of diarrhea

We identified the effects of MA at the above acupoints on the jejunal motility in rats with diarrhea. The rat model of diarrhea was generated by intragastric infusion of Folium Sennae decoction (10 mL/kg) for two days. The decoction was prepared as 10 mL containing 0.3 g crude drug. Following two days of oral gavage, the rats appeared fatigued, showed a decrease in food consumption, and suffered excessive and loose movements and feces with an unpleasant odor. The loose stool rate was approximately 49.5% of all gavaged rats, the loose stool level was 2.68, and the diarrhea index was 1.33, indicating that the model had developed appropriately.

In rats with diarrhea, the baseline amplitude and frequency of jejunal motility waves was as follows: for peristalsis waves the frequency was about 33 times/min and the amplitude about 12 cmH₂O; for translator waves the frequency was 5–6 times/min and the amplitude about 20 cmH₂O (Figure 3A). As shown in Figures 3B–D, MA at LI11 increased intrajejunal pressure from 11.95 ± 0.35 cmH₂O to 13.96 ± 0.39 cmH₂O, a significant increase of $16.82\% \pm 2.35\%$ ($P < 0.05$, $n = 17$). However, no significant change in frequency was observed (from 35.06 ± 0.19 times/min to 34.62 ± 0.15 times/min, $P > 0.05$, $n = 17$). MA at ST37 tended to increase intrajejunal pressure with no significant effect on frequency noted (intrajejunal pressure: from 12.42 ± 0.38 cmH₂O to 13.05 ± 0.38 cmH₂O, $P > 0.05$, $n = 17$).

MA at ST25 not only reduced intrajejunal pressure from 11.53 ± 0.29 cmH₂O to 6.93 ± 0.21 cmH₂O, a decrease of $39.89\% \pm 2.25\%$ ($P < 0.01$, $n = 17$), but also decreased the frequency from 33.88 ± 0.42 times/min to 22.47 ± 0.81 times/min, a significant decrease of $33.67\% \pm 2.12\%$ ($P < 0.01$, $n = 17$). MA at BL25 showed no significant effect on either intrajejunal pressure or frequency of jejunal motility (intrajejunal pressure: 11.81 ± 0.43 cmH₂O for pre-MA *vs* 11.63 ± 0.39 cmH₂O for MA; frequency: 34.09 ± 0.34 times/min for pre-MA *vs* 33.72 ± 0.33 times/min for MA). These results suggest that in rats with diarrhea, only ST25 shows any therapeutic effect. LI11 and even ST37 may induce an opposing effect or have no effect.

To further elucidate whether the above heterotopic acupoints show dual effects on jejunal motility, we also compared changes produced by acupuncture at LI11, ST37, and ST25 in normal rats and rats with constipation or diarrhea. At LI11 or ST37, the increase in intrajejunal pressure and frequency of jejunal motility were not significantly different between the normal, constipated, and diarrheic rats (intrajejunal pressure: $P = 0.16$; frequency: $P = 0.71$ in LI11; intrajejunal pressure: $P = 0.20$, and frequency: $P = 0.94$ in ST37). At ST25, the inhibition of intrajejunal pressure and frequency were not different between the above groups of rats (intrajejunal pressure: $P = 0.70$; frequency: $P = 0.48$), suggesting that acupuncture at heterotopic points may not produce dual effects on jejunal motility under physiological or pathological conditions, as with acupuncture at homotopic acupoints.

C fibers are required for the regulation of jejunal motility by acupuncture

Acupuncture modulates visceral organ function by inducing activation of the somatovisceral reflexes and changing the autonomic nervous system. Our previous studies demonstrated that modulation of gastric motility induced by acupuncture stimulation only involved the activation of fine-diameter afferent fibers, including A δ fibers and C fibers^[9]. To determine the type of afferent fibers mediating the regulation of jejunal motility by manual acupuncture, we used an A-selective demyelination agent and a C fiber blocker for this study. Firstly, cobra venom was applied for 30 min to demyelinate A fibers of the sciatic nerve, and then acupuncture at ipsilateral ST37 was administered. As shown in Figures 4A, B, and C, prior to demyelination of A δ fibers of the sciatic nerve, acupuncture at ST37 increased intrajejunal pressure significantly to $123.98\% \pm 2.07\%$ of baseline ($P < 0.05$, $n = 20$). Following demyelination of A δ fibers, acupuncture at ST37 again augmented intrajejunal pressure to $121.48\% \pm 3.06\%$ of baseline; the increase was not significantly different compared with acupuncture with no demyelination ($P > 0.05$, $n = 20$). Similarly, the frequency of jejunal motility changed from $102.12\% \pm 1.78\%$ to $95.86\% \pm 1.51\%$ of baseline ($P > 0.05$, $n = 20$).

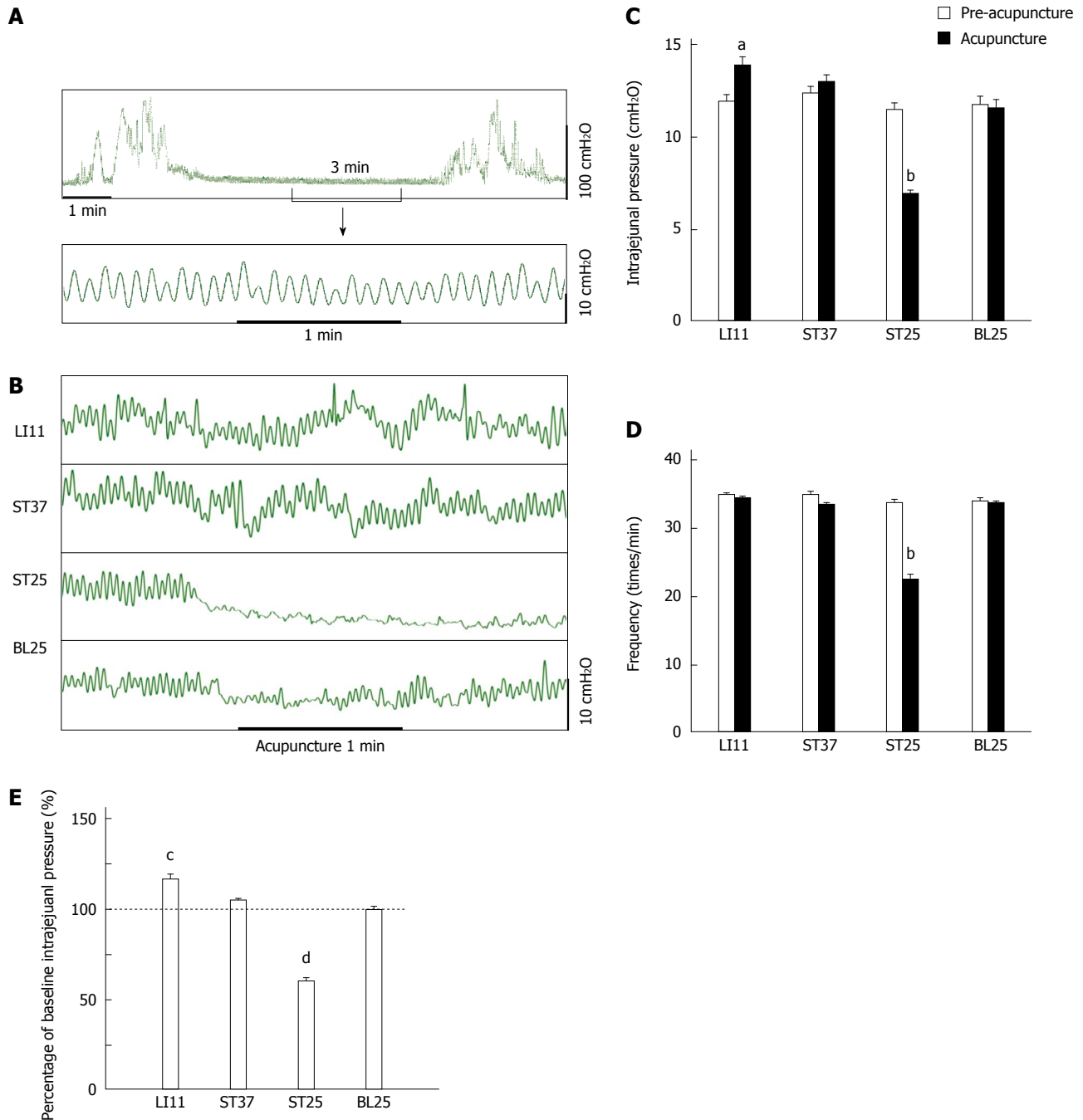


Figure 3 Effects of manual acupuncture at LI11, ST37, ST25, and BL25 on jejunal motility in diarrheic rats. A: A representative trace of jejunal motility in a rat with diarrhea; B: Representative traces of jejunal motility regulated by acupuncture at LI11, ST37, ST25, and BL25 in diarrheic rats; C: Intrajejunal pressure was significantly enhanced by acupuncture at LI11 but reduced by acupuncture at ST25 (^a $P < 0.05$, ^b $P < 0.01$ vs pre-acupuncture, paired t -test, $n = 17$); D: Only acupuncture at ST25 decreased the frequency of jejunal motility (^b $P < 0.01$ vs pre-acupuncture, paired t -test, $n = 17$); E: The percent change in intrajejunal pressure caused by acupuncture at LI11, ST37, ST25, and BL25 (^c $P < 0.05$, ^d $P < 0.01$ vs baseline, paired t -test, $n = 17$). The intrajejunal pressure was normalized by baseline, which is denoted by the dashed line.

of baseline, which was not significant different ($P > 0.05$, $n = 20$), suggesting that A δ fibers may mediate, but do not play a critical role in the regulation of jejunal motility by acupuncture.

To identify a role of C fibers in the regulation of jejunal motility by acupuncture, as in a previous report^[27], we applied capsaicin to diminish the activity of C fibers of the sciatic nerve. Figures 5A, B, and C show that acupuncture at ST37 significantly enhanced intrajejunal

pressure to $125.55\% \pm 2.15\%$ of baseline in intact rats ($P < 0.01$, $n = 20$). Following treatment with capsaicin (2%, 25 μ L) for 24 h, acupuncture at ipsilateral ST37 generated $106.63\% \pm 1.26\%$ of basal intrajejunal pressure, which was significantly different when compared pre-capsaicin treatment ($P < 0.05$, $n = 20$). However, capsaicin did not change the frequency of jejunal motility significantly (prior to C fibers inactivation: $99.92\% \pm 1.13\%$ of baseline; following C fiber inactivation:

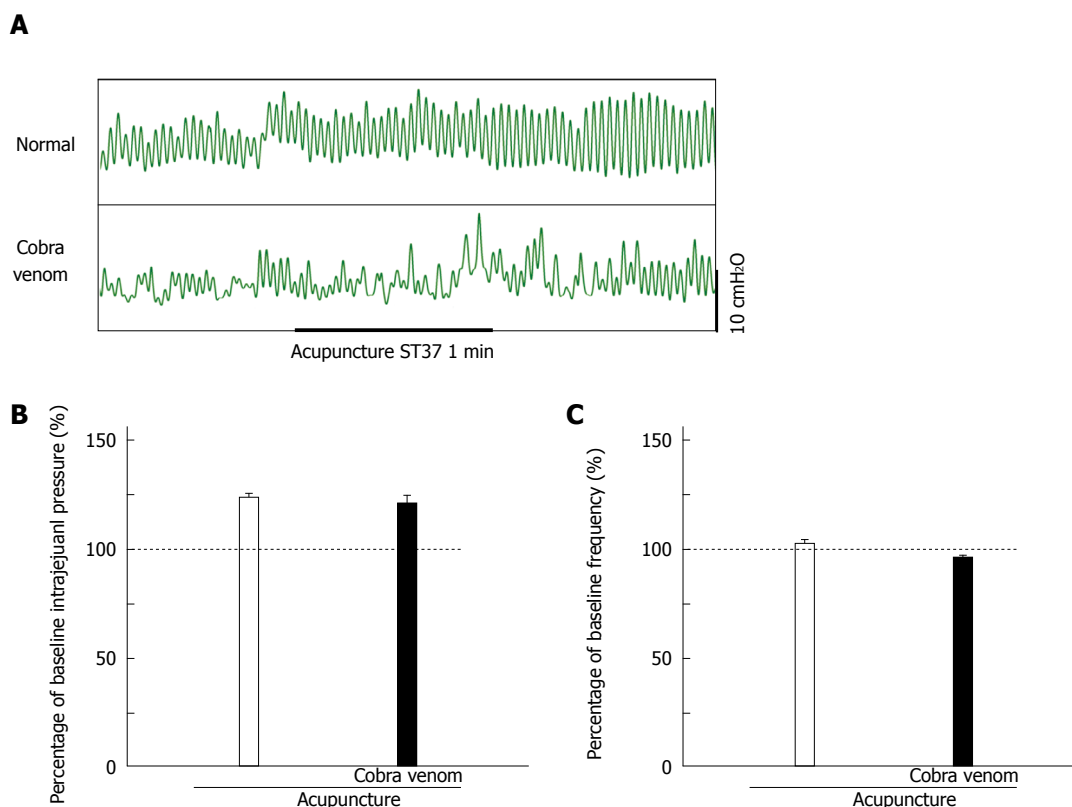


Figure 4 Effect of cobra venom on the regulation of jejunal motility by manual acupuncture at ST37. A: Representative traces of jejunal motility without and with cobra venom; B: Cobra venom did not inhibit the improved intrajejunal pressure caused by acupuncture at ST37 (unpaired *t*-test, *n* = 20). Intrajejunal pressure was normalized by baseline, shown as the dashed line; C: Cobra venom had no effect on ST37 acupuncture-mediated regulation of frequency of jejunal motility (unpaired *t*-test, *n* = 20). Frequency was normalized by baseline, as denoted by the dashed line.

99.24% \pm 0.85% of baseline, *P* > 0.05, *n* = 20). These results suggest that C fibers are required for the regulation of jejunal motility by acupuncture.

M₂ receptors play a role in mediating the excitatory effect of acupuncture at heterotopic acupoints on jejunal motility

Previous evidence indicated that the dominant cholinergic muscarinic receptors are the *M₂* subtype in the jejunum and *M₃* in the colon^[28,29]. Here we aimed to illustrate whether cholinergic muscarinic pathways play a specific role in mediating the effect of acupuncture on jejunal motility. Firstly, we applied methoctramine (0.5 mg/kg, i.v.), a selective *M₂* receptor antagonist. Figures 6A, B, and C show that in the presence of methoctramine, peristalsis frequency was inhibited to 55.58% \pm 3.92% of baseline (*P* < 0.01, *n* = 10) and amplitude to 44.91% \pm 3.86% of baseline (*P* < 0.01, *n* = 10). We then applied MA at LI11, ST37, ST25, and BL25 separately. As shown in Figures 6D and E, acupuncture at LI11 significantly rescued the amplitude of jejunal motility from 42.83% \pm 1.65% to 53.43% \pm 1.95% of baseline (*P* < 0.05, *n* = 10), but only slightly affected frequency, decreasing it from 54.83% \pm 2.26% to 52.87% \pm 2.18% of baseline (*P* > 0.05, *n* = 10). Acupuncture at ST37 rescued both the frequency and amplitude of jejunal motility markedly (frequency: from 56.78% \pm 2.21% to 66.64% \pm 2.05% of baseline, *P* < 0.05;

amplitude: from 45.15% \pm 2.22% to 70.51% \pm 2.34% of baseline, *P* < 0.01, *n* = 10), whereas acupuncture at ST25 further decreased both the frequency and amplitude of jejunal motility (frequency: from 59.85% \pm 2.74% to 38.15% \pm 2.17% of baseline, *P* < 0.01; amplitude: from 52.48% \pm 3.55% to 17.34% \pm 1.39% of baseline, *P* < 0.01, *n* = 10). Acupuncture at BL25 increased the motility amplitude of the jejunum notably, from 38.03% \pm 2.34% to 70.12% \pm 2.22% of baseline (*P* < 0.01, *n* = 10), but changed the frequency from 49.46% \pm 3.21% to only 57.89% \pm 2.60% of baseline (*P* > 0.05, *n* = 10).

DISCUSSION

In this study, we discovered that acupuncture at LI11 (containing afferents from C₅ spinal dorsal horn) and ST37 (L₅), which are heterotopic acupoints to the jejunum (T₉₋₁₂), increased the amplitude of peristalsis waves and enhanced jejunal motility not only in normal rats, but also in constipated and diarrheic rats. Homotopic acupoints, such as ST25 (T₁₀), decreased jejunal motility regardless of initial condition. However, BL25 stimulation did not change jejunal motility significantly in normal, constipated, and diarrheic rats. We also noted that activation of A δ fibers mediated the regulation of jejunal motility; however, C fibers play a more important role in the regulation of jejunal motility by manual acupuncture. *M₂*

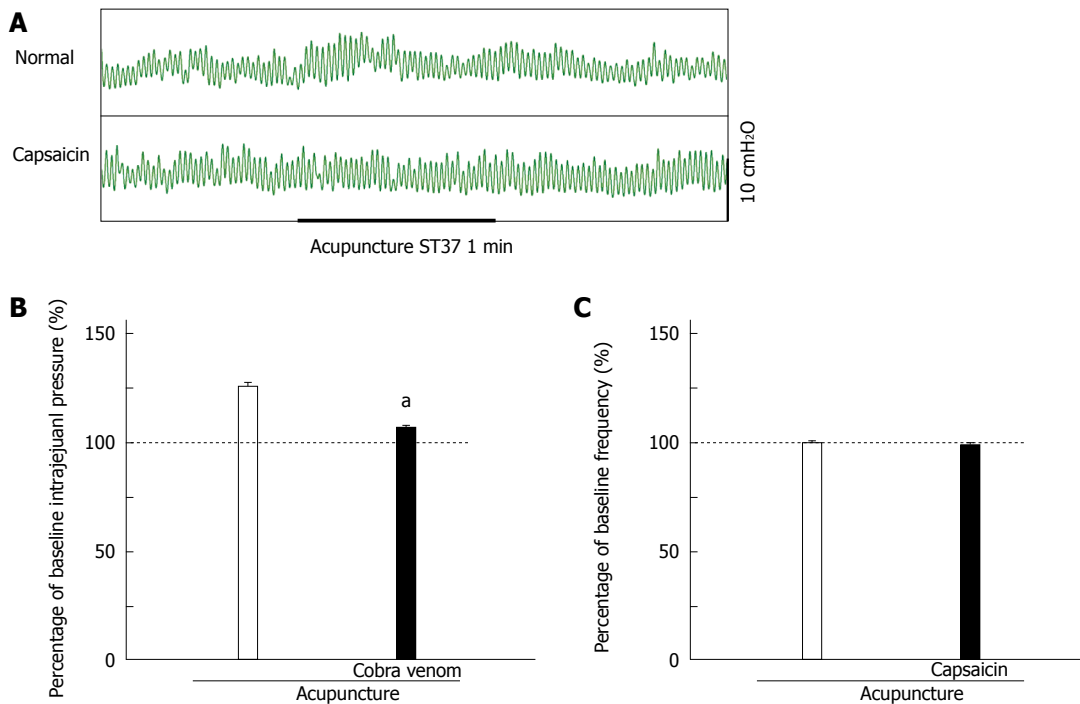


Figure 5 Effect of capsaicin on the regulation of jejunal motility by manual acupuncture at ST37. A: Representative traces of jejunal motility without and with capsaicin; B: Capsaicin significantly inhibited the increase in intrajejunal pressure caused by acupuncture at ST37 ($P < 0.05$ vs baseline, unpaired *t*-test, $n = 20$). Intrajejunal pressure was normalized by baseline, shown as the dashed line; C: Capsaicin showed no effect on ST37 acupuncture-mediated regulation of frequency of jejunal motility (unpaired *t*-test, $n = 20$). The frequency was normalized by baseline, shown as the dashed line.

receptors may mediate the enhancement of acupuncture at heterotopic acupoints in part. Therefore, our study unveiled the effects and underlying mechanism of manual acupuncture at heterotopic acupoints on jejunal motility, as well as showing that C fibers predominate in mediating the regulation of jejunal motility by acupuncture. M₂ receptors play a role in this process.

It is well known that acupuncture has regionally specific effects. Previous studies demonstrated that acupuncture at the hindlimb increased gastric motility, whereas acupuncture at the abdomen inhibited gastric motility in anesthetized rats^[8,30]. In addition to traditional acupuncture theory, in which some acupoints exhibit dual effects based on the basal condition, dual effects of EA on gastric motility have also been reported in some studies^[23-26]. For example, Tatewaki *et al.*^[24] reported that manual acupuncture at ST36 induced dual effects: stimulating gastric contractions in rats with hypomotility and inhibiting gastric contractions in rats with hypermotility. In the current study, we observed that acupuncture at heterotopic acupoints caused an excitatory effect, whereas homotopic acupoints induced an inhibitory effect on jejunal motility, no matter whether the intestines were normal, hypomotile, or hypermotile. These data suggest that acupuncture at heterotopic or homotopic acupoints does not have dual effects on jejunal motility in rats, whereas stimulation of ST37 only slightly increased intra-jejunal pressure in diarrheic rats. We would like to note that in this study, acupuncture at BL25, which is also a heterotopic acupoint, did not show any consistent effect on jejunal motility. The cause of this phenotype will be

investigated further in future research.

It has been demonstrated that somatic afferents from the skin and muscle are involved in the control of gastrointestinal motility^[10-14]. Thus, cutaneous stimulations, such as acupuncture, may stimulate the somatic afferent nerves of the skin and muscles that are important for evoking autonomic reflexes *via* the homotopic segments or outflow from the brain stem. Our previous study demonstrated that only stimulations greater than the threshold for activation of A δ and/or C fibers could profoundly modulate gastric motility^[9]. Based on the accumulated electrophysiological and pharmacological evidence, the first-components triggered by acupuncture at a short latency are the small diameter A δ fibers, with the second stimulated component being the unmyelinated C fibers. Stimulations below the threshold strength for A δ fibers do not effectively trigger regulatory effects on gastric motility regardless of the locations of the acupoints. Koizumi *et al.*^[31] conducted a systematical analysis of the relationship between the magnitude of cutaneous-intestinal reflex response and the groups of afferent fibers stimulated, and noted that stimulation of the sural afferent nerves of the hindlimb elicited facilitative jejunal reflexes, which were obtained when the stimulus intensity activated group III fibers, and maximal facilitation was obtained when the stimulus intensity activated group IV afferent fibers. Stimulating groups III-IV, particularly group IV, or C afferent fibers of the hindlimb caused an excitatory intestinal reflex response. In contrast, stimulation of group IV abdominal nerves only inhibited the intestinal reflex response. These data suggested that only

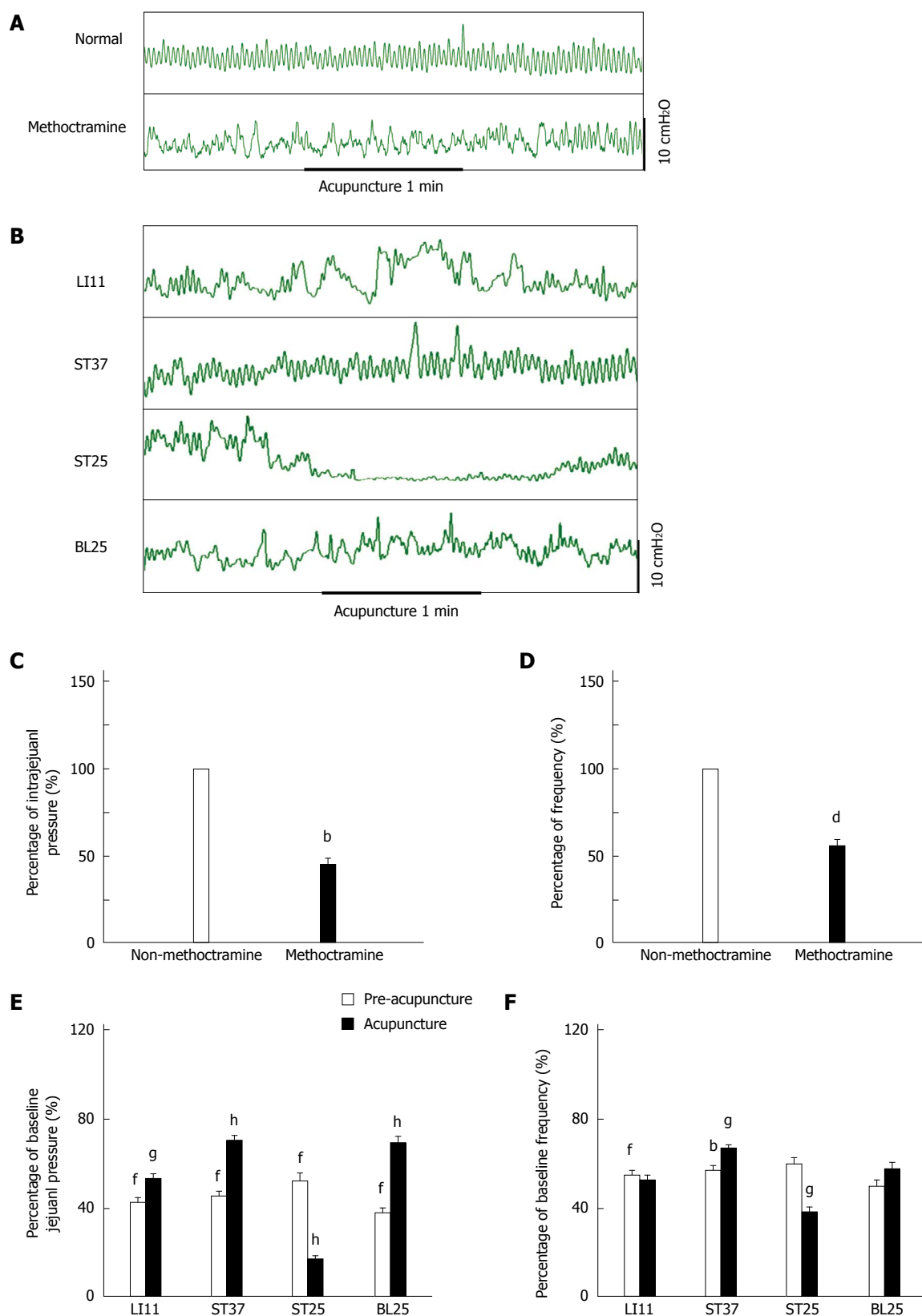


Figure 6 Effect of methoctramine on the regulations of jejunal motility by manual acupuncture at LI11, ST37, ST25, and BL25. **A:** Representative traces of jejunal motility without and with methoctramine; **B:** Representative traces of the regulations of jejunal motility by acupuncture at LI11, ST37, ST25, and BL25 separately following the administration of methoctramine; **C:** Methoctramine reduced the intrajejunal pressure significantly ($^bP < 0.01$ vs baseline, unpaired *t*-test, $n = 10$); **D:** Methoctramine decreased the frequency of jejunal motility significantly ($^dP < 0.01$ vs control, unpaired *t*-test, $n = 10$); **E:** Acupuncture at LI11, ST37, and BL25 rescued the methoctramine-mediated inhibition of intrajejunal pressure significantly, but acupuncture at ST25 further decreased intrajejunal pressure in the presence of methoctramine ($^fP < 0.01$ vs non-methoctramine; $^gP < 0.05$, $^hP < 0.01$ vs pre-acupuncture, paired *t*-test, $n = 10$). Intrajejunal pressure was normalized by baseline without any treatment, the dashed line denotes basal intrajejunal pressure without methoctramine; **F:** Only acupuncture at ST37 significantly rescued the methoctramine-mediated inhibition of frequency of jejunal motility ($^fP < 0.01$ vs non-methoctramine, shown as the dashed line; $^gP < 0.05$, $^hP < 0.01$ vs pre-acupuncture, paired *t*-test, $n = 10$). Frequency was normalized by baseline without any treatment. The dashed line denotes basal frequency of jejunal motility without methoctramine.

acupuncture stimulation of an intensity strong enough to excite A δ (or group III) and/or C (or group IV) afferent fibers may induce apparently excitatory/inhibitory modulation of gastrointestinal motility. Previous studies also showed that manual acupuncture activates I, II, III, and IV afferent fibers^[32-34]. As described previously^[33], in this paper, stimulation by manual acupuncture manipulation at an intensity of 2 Hz is sufficient to activate A δ and C fibers. Our data also demonstrate that although stimulating A δ afferent fibers *via* manual acupuncture may enhance jejunal motility, stimulation of C fibers produces a more significant excitatory jejunal reflex response.

Muscarinic acetylcholine receptors comprise five distinct subtypes (M₁₋₅) and are widely distributed in smooth muscle throughout the body, including the gastrointestinal tract^[35-37]. In gastrointestinal smooth muscles, M₂ and M₃ muscarinic receptor subtypes are preferentially expressed^[35]. The recent use of mutant mice lacking specific muscarinic receptor subtypes has revealed that not only M₃ but also M₂ receptors may play a direct role in inducing contraction in gastric and ileal smooth muscles^[38-42]. Both M₂ and M₃ receptors mediate contractions induced by the stimulation of cholinergic nerves^[43]. M₂ and M₃ receptors induce smooth muscle contractions *via* the activation of G proteins of the G_i and G_q family, respectively^[42]. M₂ receptors are less active than M₃ receptors in mediating cholinergic contractions in wild-type tissues. Although the mechanism is unknown, M₂ receptor activity is reduced when M₂ and M₃ receptors are activated simultaneously. It has been demonstrated that cholinergic contractions *in vitro* are mediated predominantly by M₃ receptors. In contrast, stimulation of the most abundant subtype M₂^[44,45] has been suggested to exert minor indirect contraction by reversing histamine- or forskolin-induced relaxation^[46-48]. However, a recent study verified that small but significant contractions remained in the homozygous muscles (bladder, 5%; ileum, 23%-28%) and that M₂ receptors mediated the residual contraction of M₃ homozygous muscles directly^[49]. Regardless of direct or indirect involvement in the contraction of intestinal muscles, in the current study, methoctramine inhibited jejunal motility significantly, suggesting that M₂ receptors mediate the contraction of jejunal muscle. However, acupuncture at the heterotopic acupoints LI11 and ST37 rescued in part the methoctramine-induced decrease in jejunal motility, suggesting that M₂ receptors may mediate some jejunal contraction by acupuncture. M₃ receptors may play an important role in rescuing jejunal contraction by acupuncture despite its lower density in jejunal tissue. Our data in M₂/M₃ knockout mice have also demonstrated that both M₂ and M₃ receptors mediated increased jejunal motility by acupuncture at heterotopic acupoints (data not shown).

In summary, we conclude that acupuncture at heterotopic acupoints, particularly in the limbs, increases the amplitude of jejunal motility regardless of whether the intestine showed normal motility, hypomotility, or hyper-

motility. C afferent fibers are essential for this enhancing effect of acupuncture on jejunal motility; however, A δ afferent fibers may mediate the transduction of the acupuncture signal. Cholinergic muscarinic receptors, including M₂ and M₃ subtypes, play an important role in the enhancement of jejunal motility by acupuncture at heterotopic acupoints.

COMMENTS

Background

Previous studies identified an effect of somatic stimulation on gastrointestinal motility under normal conditions. However, little effort has been made to investigate the effect of acupuncture on small intestinal motility, particularly under pathological conditions, and the precise mechanism of action of acupuncture with respect to small intestinal motility remains elusive.

Research frontiers

Acupuncture at heterotopic acupoints may improve gastrointestinal motility under normal conditions. Previous investigations rarely discussed the effects of acupuncture on small intestinal motility under pathological conditions. Only stimulation intensities above the threshold for activating A δ and/or C fibers modulate gastrointestinal motility. The dominant cholinergic muscarinic receptors in the jejunum are of the M₂ subtype. Here, we hypothesized that acupuncture at heterotopic acupoints may regulate jejunal motility by activating A δ and/or C fibers, and that M₂ receptors mediate this effect.

Innovations and breakthroughs

In this study, the authors show an enhancement of jejunal motility by manual acupuncture at heterotopic acupoints regardless of initial condition. The underlying mechanism of action is that C fibers predominantly mediate the regulation of jejunal motility by acupuncture, and M₂ receptors play a role in this process.

Applications

In view of the fact that acupuncture at heterotopic acupoints increases jejunal motility regardless of initial condition and that C fibers are involved in regulating acupuncture-induced jejunal motility, this study may aid acupuncture practitioners select acupoints and stimulation intensities for the treatment of patients with gastrointestinal disorders. The fact that M receptors are involved may aid in developing drugs for application in gastrointestinal disorders.

Terminology

Heterotopic acupoint are points where the afferents originate in different spinal segments from the efferents that innervate the visceral organs. Homotopic acupoints are points where the afferents originate in the same segment as the efferents that innervate the visceral organs.

Peer review

The authors may want to directly state the primary outcomes and method of analysis for primary outcomes rather than practicing them in separate paragraphs.

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