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RAPID COMMUNICATION

Effect of electroacupuncture at Sibai on the gastric myoelectric acitivities of denervated rats

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Abstract

AIM: To explore the mechanism of the exciting effects of electro-acupuncture (EA) at Sibai on the gastric myoelectric activities.

METHODS: A total of 32 rats were randomly divided into four groups. Through intraperitoneal injection with atropine (the anti-cholinergic agent by blockade of muscarinic receptors), hexamethonium (automatic nerve ganglion-blocking agent) and reserpine (anti-adrenergic agent by depleting the adrenergic nerve terminal of its norepinephrine store), effects of EA at Sibai on the gastric myoelectric activities of the denervated rats were observed.

RESULTS: After intraperitoneal injection of atropine and hexamethonium, the average amplitude and ratio of period to time in the phase of high activity of gastric myoelectric slow wave, and the average numbers of the peaks of gastric myoelectric fast wave were significantly decreased (P < 0.01, P < 0.05, P < 0.01), while after intraperitoneal injection of reserpine, the aforementioned three parameters were increased (P < 0.01, P < 0.05, P < 0.01). EA at Sibai point partially relieved the inhibitory effect of atropine and hexamethonium on the gastric myoelectric activities in the rats (P < 0.05 or P > 0.05).

CONCLUSION: Cholinergic and adrenergic nervous systems and autonomic nerve ganglion participate in the peripheral passage of the controlling effects of EA at Foot Yangming Channel on gastrointestinal tract.

Key words: Electro-acupuncture; Rats; Sibai; Nerve block; Gastric myoelectric activities

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INTRODUCTION

Electroacupuncture (EA) at certain points always exhibits marked effects (inhibitory or stimulatory) on organ activities. Our previous studies indicated that EA at the Sibai point exhibited exciting effects on gastric myoelectric activities. These stimulant actions appeared on average amplitude of high activities of slow waves, the ratio between the time-course of high activities of slow waves and cycle of slow waves, average frequency of fast waves. No apparent stimulation was observed at the control point after EA. These results suggest that there are inherent specific correlations between Channels and zang and fu organs. The aim of this study was to investigate the mechanism underlying the exciting effects of EA at Sibai on gastric myoelectric activities. To this purpose, atropine (the anti-cholinergic agent by blockade of muscarinic receptors), hexamethonium (automatic nerve ganglion- blocking agent) and reserpine (anti-adrenergic agent by depleting the adrenergic nerve terminal of its norepinephrine store) were used before EA at Sibai. Gastric myoelectric activities of the rats with nerve blockade were observed[2-4].

MATERIALS AND METHODS

Animals and experimental design

Healthy adult rats (n = 32, female or male, body mass: 200-300 g) were randomly divided into four groups (n = 8 per group): (1) EA at Sibai plus saline group: EA at Sibai for 30 min and simultaneously recorded gastric myoelectric activities for 60 min as baseline (control); EA repetition and recording after intraperitoneal injection (ip) with 2 mL saline. (2) EA at Sibai plus atropine (ip) group: EA and recording gastric myoelectric activities same as the first group. (3) EA at Sibai plus hexamethonium (15 mg/kg, ip) group: EA and recording gastric myoelectric activities same as the first group. (4) EA at Sibai plus reserpine (0.5 mg/kg, ip 24 h and 48 h prior to EA) group: EA and

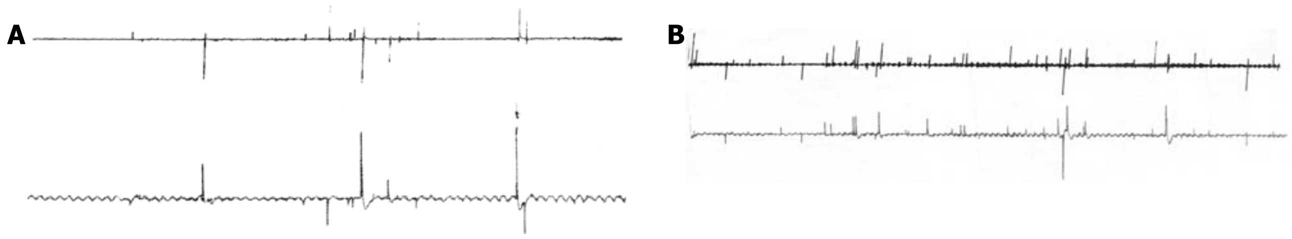

Figure 1 Slow and fast wave of the rats' gastric myoelectric activities before and after EA at Sibai. **A**: Before EA; **B**: After EA.

recording gastric myoelectric activities same as the first group.

Acupuncture points location and parameters for EA stimulation

The acupuncture points were chosen and located according to Lin's "Experimental Acupuncture" - "diagram of acupuncture points for experimental animals"[1]. Comparative anatomic methods were also used as reference to locate the acupuncture points on rat. Parameters for EA stimulation, namely slow and fast waves (slow wave: 4 Hz; fast wave: 50 Hz), impulse width 0.5 ms, output voltage 2-4 V, output electricity 4-6 mA, 0-60 peak altitude (1 KΩ loaded), slight shake of the needle, were determined as threshold intensity. Stimulation time 30 min and output stimulation intensity were adjusted every 10 min interval to maintain proper intensity.

Placement of gastric electrode

Rats were deprived of food but not water 24 h prior to experiments. Rats were weighed and anesthetized with 10% urathe 0.5 mL (100 mg, ip), tightened and faced up. Abdomen was opened along the middle line. One platinode (diameter 2 mm) was placed under the gastric sinus portion and approximately 0.5 cm to the pyloric sphincter. The other platinode was placed on the body of the stomach and close to the first platinode, 1 cm. The cables were leaded to the skin and tightened with a plastic tube around the neck. Penicillin was injected to prevent infection. The experiments were processed after 7 d.

Recording of gastric myoelectric activities

Rats were deprived of food but not water 24 h prior to experiments. Rats were weighed and anesthetized with 10% urathe 0.5 mL (100 mg ip). Two cables were connected with the front amplifier of the two channels of the physiological recorder. Fast wave and slow wave were recorded simultaneously. Recording parameters for fast wave were: time constant 0.02 s, high frequency filter 30 Hz, and sensitivity 1 mV/cm. Recording parameters for the slow wave were: time constant 2 s, filtration 10 Hz, sensitivity 5 mV/cm. Paper speed was 10 mm/min.

Measurement of fast wave and slow waves of gastric myoelectric activities

Three minutes of wave shape, regularity, and a maximum of wave width of the slow wave were defined as one section; four such sections were accumulated and calculated the average amplitude of one section. Cycle of slow wave was chosen in at least 3 sections. The cycle period and time courses of the high activity phase were measured, and the ratio of these two measurements were calculated. Consecutive 10 min of all fast waves were defined as one section; three such sections were accumulated and calculated the average frequency (time/min) of one section. The difference between these two measurements which treated before and after was also calculated.

Statistical analysis

Data were expressed as mean ± SD. Paired t test was used to compare the difference before and after treatment within the same group. The difference among groups was analyzed by one-way ANOVA or q' test. All the data were processed with SPSS11.5 software.

RESULTS

Effect of EA on average amplitude of high activity phase of slow wave in anesthetized rats

There was no significant difference in average amplitude of high activity phase of slow wave among groups after EA. Both atropine and hexamethonium attenuated the average amplitude of high activity phase of slow wave ($P < 0.01$ *vs* before blocking agent; $P < 0.01$ *vs* Sibai plus saline group) (Figure 1). EA markedly abolished the inhibition induced by both atropine and hexamethonium and restrained the activity ($P < 0.05$ *vs* blocking agents alone). Reserpine obviously increased the average amplitude of high activity phase of slow wave ($P < 0.01$ *vs* before blocking agent; $P < 0.01$ *vs* Sibai plus saline group). EA at Sibai still enhanced the average amplitude even after pretreatment with reserpine ($P < 0.05$ *vs* (1) after EA) (Table 1, Figure 1).

Effect of EA on the ratio between time courses of high activity of slow wave and slow wave cycle in anesthetized rats

As shown in Table 2, there was no marked difference in the ratio between time courses of high activity of slow wave and slow wave cycles in anesthetized rats. Both atropine and hexamethonium decreased the ratio ($P < 0.05$ *vs* (1) after EA). EA restrained the inhibition and recovered the ratio to the prior value before using blocking agent ($P > 0.05$). Reserpine slightly increased the ratio without significant difference compared to the value before using blocking agent. EA at Sibai after reserpine application markedly enhanced the ratio ($P < 0.05$) (Table 2).

Table 1 Effect of EA on amplitude of high activity phase of slow wave in rats (mean ± SD, mV)

Group	(1) After EA	(2) Blocking agent	(3) Blocking agent + EA	(2)-(1)	(3)-(1)
Sibai + saline	5.35 ± 0.32	5.21 ± 0.76	5.38 ± 0.26	0.14 ± 0.32	0.03 ± 0.09
Sibai + atropine	5.31 ± 0.44	2.38 ± 0.71[b]	5.21 ± 0.54[a]	2.93 ± 0.35[d]	0.10 ± 0.11
Sibai + hexamethonium	5.28 ± 0.48	2.65 ± 0.66[b]	5.16 ± 0.62[a]	2.63 ± 0.38[d]	0.12 ± 0.10
Sibai + reserpine	5.34 ± 0.62	5.55 ± 0.52[a]	5.61 ± 0.57[a]	0.59 ± 0.21[c]	0.27 ± 0.22[c]

[a]$P < 0.05$, [b]$P < 0.01$ *vs* (1) after EA; [c]$P < 0.05$, [d]$P < 0.01$ *vs* Sibai plus saline group.

Table 2 Effect of EA on the ratio between time courses of slow wave and slow wave cycle in rats (mean ± SD, mV)

Group	(1) After EA	(2)Blocking agent	(3) Blocking agent + EA	(2)-(1)	(3)-(1)
Sibai + saline	0.49 ± 0.04	0.48 ± 0.04	0.48 ± 0.04	0.01 ± 0.01	0.00 ± 0.01
Sibai + atropine	0.47 ± 0.05	0.46 ± 0.04[a]	0.48 ± 0.05	0.01 ± 0.01	0.01 ± 0.01
Sibai + hexamethonium	0.48 ± 0.06	0.47 ± 0.04[a]	0.48 ± 0.06	0.01 ± 0.01	0.01 ± 0.01
Sibai + reserpine	0.48 ± 0.06	0.49 ± 0.06	0.49 ± 0.05[a]	0.01 ± 0.01[c]	0.01 ± 0.01

[a]$P < 0.05$ *vs* (1) after EA; [c]$P<0.05$ *vs* Sibai plus saline group.

Table 3 Effect of electroacupuncture (EA) on average frequency of fast wave in anesthetized rats (mean ± SD, mV)

Group	(1) After EA	(2) Blocking agent	(3) Blocking agent + EA	(2)-(1)	(3)-(1)
Sibai + saline	36.90 ± 10.95	36.11 ± 9.55	36.32 ± 9.69	0.79 ± 0.98	0.58 ± 0.92
Sibai + atropine	36.38 ± 11.77	34.33 ± 10.63[b]	36.08 ± 11.32	2.05 ± 0.99[c]	0.30 ± 0.88
Sibai + hexamethonium	37.04 ± 11.08	35.21 ± 10.23[b]	36.65 ± 11.12	1.83 ± 0.90[c]	0.39 ± 0.94
Sibai + reserpine	37.34 ± 9.66	38.24 ± 10.14[a]	38.33 ± 11.13[a]	0.90 ± 0.92	1.01 ± 0.90

[a]$P < 0.05$, [b]$P < 0.01$ *vs* (1) after EA; [c]$P < 0.05$ *vs* Sibai plus saline group.

Effect of EA on average frequency of fast wave in anesthetized rats

No significant change in the average frequency of fast wave was observed among groups after EA practice. Atropine and hexamethonium significantly reduced the average frequency of fast wave ($P < 0.01$ *vs* (1) after EA). The decreased average frequency induced by atropine or hexamethonium also appeared different with the saline group ($P < 0.05$). EA at Sibai slightly increased average frequency of fast wave, but did not reach the significance. Rats pretreated with reserpine exhibited increased average frequency ($P < 0.05$ *vs* (1) after EA) (Table 3).

DISCUSSION

"Twelve channels connect with arms and body surface outwardly and associate with zang and fu organs inwardly". This theory has been widely recognized in channel theory and zang/xiang quotation of acupuncture, one of the branches of traditional Chinese medicine. The points on the body surface deeply correlate with zang and fu organs. "Following channels to select points" - this theory has been adapted in clinical practices. Experiments and clinical practices indicate that electroacupuncture at some points exhibits significant effects on certain organs. The subsequent challenge is how to clarify the mechanism underlying the correlation between points and organ activities. Nerve system and endocrine system dominate most of the organ function and activities. The role of the nerve system in gastric myoelectric activities induced by electroacupuncture at Sibai is the main topic in this study.

Atropine is a typical anti-cholinergic agent, which acts through blockade of muscarinic receptors. Hexamethonium is a ganglion-blocking agent. Reserpine has been used to address the role of adrenergic nerve in physiological study; and it exhibits anti-adrenergic effect by depleting norepinephrine storage in the terminals. For this study, atropine, hexamethonium, and reserpine were administered by intraperitoneal injection to rats. Our results showed that both atropine and hexamethonium abolished the increased gastric activities induced by EA. The gastric activities were inhibited after both atropine and hexamethonium pretreatment. Average amplitude of high activity phase of slow wave, the ratio between time-courses of high activity phase of slow wave and slow wave cycle, and average wave frequency of fast wave were significantly reduced. These results suggest that cholinergic and sympathetic nerves are involved in the regulation induced by EA at point of Foot Yangming Channel. EA at Sibai exhibited strong exciting effects and antagonized the inhibition induced by both atropine and hexamethonium. These effects included enhanced average amplitude of high activity phase of slow wave, the ratio between time-courses of high activity phase of slow wave and slow wave cycle, and average wave frequency of fast wave. Thus, we can draw an important conclusion that both postganglionic cholinergic nerve and preganglionic sympathetic nerve are involved in the gastric exciting effects induced by EA at Sibai. Meanwhile, both atropine and hexamethonium only partially inhibited the gastric activities. These experiments suggested that a third "no cholinergic, no adrenergic" nerve fiber may participate to control gastric myoelectric activities. Peptide nerve may mediate the gastric myoelectric activities. EA at Sibai may encourage some peptide transmitters release and regulate gastric activities. It has been reported that peptides are related to gastrointestinal mobility[5,6,8]. EA at Zusanli, Tiansu, and Liangmeng of Foot Yangming Channel increased the complex mobility of gastric sinus portion, small intestine and closed empty intestine. Motilin, gastrin and substance P concentrations were increased. Acupuncture can incompletely abolish the inhibitory action on gastric motility induced by atropine[7,9,10]. EA at Zusanli point of Foot Yangming Channel exhibits exciting effects on gastric myoelectric activities (amplitude, time-course, and frequency of wave) in anesthetized rats. Intravenous injection with atropine inhibits these effects[11-14]. Contrast with this result, EA at Zusanli exhibits opposing effects on gastric myoelectric activities in awaken rabbits: inhibition of gastric mobility, gastric myoelectric frequency, and wave amplitude[15,16]. Some authors suggest that the inhibition induced by EA at Zusanli on gastric activities is not inhibitory. The baseline control of gastric myoelectric activities plays an important role in the regulation of EA[14,17,18]. EA leads to exciting effects for low gastric activities and inhibitory effects for high gastric activities. This phenomenon has been recognized as "Body-Organ" reflection[19-21]. Most experiments proved that nerve-body fluid factors are involved in the effects of EA at points of Foot Yangming on gastric mobility function. Related experiments indicate that the inhibition of gastric mobility can be restrained by acupuncture at different points (head, body, and lower limb) of Foot Yangming Channel. Substance P, motilin, gastrin, and growth-inhibitory factors in gastric sinus section and medulla oblongata vary correspondingly. Acupuncture at Foot Yangming Channel regulates gastric mobility possibly through activating and releasing brain intestinal peptide (BIP) from central and local areas[22-24]. Atropine reduces gastric myoelectric activities and BIP release. Acupuncture at Zusanli partially relieves the inhibitory effect and BIP content. These results indicated that peptide nerve may be involved in the efferent portion of acupuncture. Motilin, gastrin and substance P *etc.* released from peptide nerve participated in the process in which EA restored the inhibited gastric myoelectric activities to normal properties[25-27], which is consistent with our present study.

Reserpine is an adrenergic nerve-blocking agent, which acts by depleting the adrenergic nerve terminal of its norepinephrine and 5-HT transmitters store[28,29]. The gastric mobility was enhanced after the intraperitoneal injection of reserpine, suggesting that adrenergic nerve is involved in the gastric myoelectric activities regulation. Catecholamine participates in this process[30]. Effects of acupuncture on gastric activities are related to domination of the vagus nerve and other exciting nerves on the basis of denervation of adrenergic action.

In conclusion, cholinergic and adrenergic nervous systems and autonomic nerve ganglion participate in the peripheral passage of the controlling effects of EA at the Foot Yangming Channel on gastrointestinal tract.

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