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BASIC RESEARCH

Effects of electroacupuncture on cardiac and gastric activities in acute myocardial ischemia rats

Shu-Bin Wang, Shu-Ping Chen, Yong-Hui Gao, Ming-Fu Luo, Jun-Ling Liu

Shu-Bin Wang, Department of Acu-moxibustion, General Hospital of Coal, Beijing 100028, China
Shu-Ping Chen, Yong-Hui Gao, Jun-Ling Liu, Department of Physiology, Institute of Acu-moxibustion, China Academy of Chinese Medical Sciences, Beijing 100700, China
Ming-Fu Luo, Department of Anatomy, Institute of Acu-moxibustion, China Academy of Chinese Medical Sciences, Beijing 100700, China
Author contributions: Wang SB, Chen SP, Gao YH performed the experiments; Wang SB made the Chinese draft; Liu JL designed the research, wrote and revised the paper in both Chinese and English; Chen SP contributed to data-analysis and plotting; Luo MF provided the technical assistance to NOS histochemical staining of tissue samples.
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Correspondence to: Jun-Ling Liu, Department of Physiology, Institute of Acu-moxibustion, China Academy of Chinese Medical Sciences, 16 Nanxiaojie, Dongzhimennei, Beijing 100700, China. jlliu2765@yahoo.com.cn
Telephone: +86-10-64014411-2765 Fax: +86-10-84046331
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Abstract

AIM: To observe the effect of electroacupuncture (EA) of "Neiguan" (PC6) and "Gongsun" (SP4) on pathological changes of the heart and stomach in rats with acute myocardial ischemia (AMI), and to explore its underlying mechanism.

METHODS: Fifty Wistar rats were randomized into control, model, PC6, SP4 and PC6 + SP4 groups (n = 8 each group). An AMI model was established by occlusion of the descending anterior branch (DAB) of the left coronary artery. ECG-ST of cervico-thoracic lead and electrogastrogram (EGG) were recorded. EA was applied to PC6, SP4 and PC6 + SP4 groups, respectively. At the end of experiments, the rats were transcardically perfused with 4% paraformaldehyde, and the heart base myocardium, gastric antrum and duodenum tissues were sampled, sectioned and stained with a reduced form of nicotinamide-adenine dinucleotide phosphate (NADPH)-diaphorase histochemical method for displaying nitric oxide synthase (NOS) activity.

RESULTS: After AMI, ECG-ST values elevated. After EA, the elevated ECG-ST values at 20 min in PC6 group, at 30 min in PC6 + SP4 and SP4 groups had no significant differences in comparison with their respective basal values before AMI. Following AMI, the amplitude and frequency of slow waves of EGG decreased remarkably ($P < 0.05$). At 30 min after EA, the mean amplitude and frequency of slow waves of EGG in the three EA groups had no marked differences compared with their individual basal levels and those in the control group. After AMI, the mean integral grey values of NOS-positive product in myocardium, gastric antrum and duodenum tissues in the model group increased remarkably in comparison with the control group, while those in three EA groups were lower than those in the model group. No significant differences were found in ECG-ST and EGG improvement among the three EA groups. However, EA of PC6 had a better effect on ECG-ST and EA of PC4 had a better effect on EGG, respectively.

CONCLUSION: EA of PC6, SP4 and PC6 + SP4 can significantly promote the recovery of cardiac and gastric electrical activities after AMI, and up-regulate NOS expression in myocardium, gastric antrum and duodenum tissues.

Key words: Electroacupuncture; Myocardial ischemia; ECG-ST; Electrogastrogram; Heart base; Gastric antrum; Duodenum; Nitric oxide synthase expression

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INTRODUCTION

It has been well documented that acupuncture of Neiguan (PC6) can effectively improve symptoms of angina, palpitation, *etc* and the left cardiac function in coronary heart disease (CHD) patients[1-4]. Experimental studies in rabbits, rats, and cats also showed that acupuncture improves myocardial ischemia (MI), hemodynamics, microcirculation and energy metabolism of ischemic heart, decreases susceptibility to ventricular

tachycardia, and protects myocardiocytes from injury[5-12]. PC6 combined with other acupoints often functions well in relieving pregnancy or MI-induced vomiting, alimentary canal ulceration, abdominal pain, *etc* and regulating secretion activities of various hormones in the gastrointestinal tract[13-15]. PC6 combined with Gongsun (SP4) can improve electrocardiogram (ECG) of CHD patients[16], and lessen gastrointestinal reactions in cancer patients undergoing chemotherapy[17,18]. It was reported that after injection of choleratoxin B coupled horseradish peroxidase (HRP) in PC6 and simultaneous electroacupuncture (EA) stimulation of SP4, the number of the labeled neurons in C6-C8 spinal ganglia and dorsal horns increases significantly and the distribution segments are expended[19], displaying a synergistic action. However, majority of these studies only explored the effect of acupuncture of a single acupoint on one internal organ and fewer studies have been conducted to explore its effect of one or more acupoints on two-visceral activities. For this reason, the present study was designed to observe the characteristics and regularities of EA of PC6 and/or SP4 in regulating the cardiac and gastric functional activities in acute myocardial ischemia (AMI) rats, and to analyze its underlying mechanisms.

MATERIALS AND METHODS

Animals and grouping

Forty male Wistar rats (weighing 250-320 g), supplied by the Experimental Animal Center of Chinese Academy of Medical Sciences, were used in the present study. All the rats fasted for about 24 h before each experiment (with free access to water) and were randomized into control, model, PC6, SP4 and PC6 + SP4 groups (n = 8 in each group). All procedures were approved by the Administrative Committee of Laboratory Animal Care and Use of Chinese Academy of Medical Sciences.

Main instruments and reagents

Instruments used in this study included multi-channel physiological recorder (RM-6000 Nihon Kohden, Japan), PowerLab data acquisition system (Australia, AD/instruments), mini-animal respirator (Jiangwan Type-Ⅱ), HANS EA apparatus (LH202H), DRB-2D electrical blanket, cryostat freezing microtome (Leica, Leitz1720), light microscope (OLYMPUS), image analyzer (MSP UV-VIS 2000, USA).

Reagents used in this study were a reduced form of nicotinamide-adenine dinucleotide phosphate (NADPH), nitroblue tetrazolium (NBT, Sigma), paraformaldehyde (Sigma).

Experimental protocols

Surgical operation: After anesthesia with a mixture solution (0.5 mL/100 g, ip) of 1.5% chloralose (5 mg/100 g) and 25% urethane (42 mg/100 g), the rat was fixed on an animal table for performing trachea cannula, thoracotomy between the 3rd and 4th intercostal spaces on the left side of the chest along the sternum, and laparotomy below the xiphoid-process.

Establishment of AMI model: After turning on artificial respirator (60 cycles/min), a surgical suture (gauge-0) was put through the myocardium beneath the root part of the descending anterior branch (DAB) of the left coronary artery for inducing AMI.

Electrogastrogram (EGG) recording electrode resettlement: For EGG recording, a pair of stainless steel electrodes was implanted beneath the subserosa about 0.5 cm apart from the pylorus of gastric antrum and a reference electrode was placed subcutaneously in the adjoining incision.

Electrocardiogram (ECG) recording electrode resettlement: For recording ECG of the cervico-thorax lead, two stainless steel electrodes were placed beneath the skin close and left to the xiphoid-process, and the neck back, respectively, and the reference electrode was placed beneath the skin of the right hindlimb.

EA: Bilateral PC6 and SP4 were located as previously described[20]. Two acupuncture needles (Gauge-28, 0.5 cm) were separately inserted into each acupoint, and connected to the HANS EA apparatus to stimulate the acupoint for 30 min with parameters of 2/15 Hz and 1-3 mA (increased gradually).

Procedure of management: After settlement of all the electrodes, ECG and EGG were recorded using polygraph, Power-Lab/8S-Chart 6.0 and computer before and during occlusion of DAB (30 min) and reperfusion (AMI/R, 30 min). EA was given just before the occlusion.

Sampling: After physiological experiments were finished, the animals were subjected to transcardial perfusion with normal saline (300 mL), and 4% polyoxymethylene in a phosphate buffer (4℃, 400 mL, 0.1 mol/L, pH 7.4), respectively. The left-atria tissue of the cardiac base (between the aorta and the pulmonary artery, containing nerve plexus controlling cardiac functional activities), gastric antrum and annectant duodenum tissues were taken and equilibrated in 20% sucrose overnight, and stored at 4℃.

Staining and observation of tissue samples: Tissue samples were cut into 20-μm thick sections and mounted onto gel-treated glass slides. The sections were washed twice with PBS and incubated to demonstrate NADPH-diaphorase activity in the following medium: 0.1 mol/L phosphate buffer solution (pH 7.4, 10 mL) containing 5 mg of NBT, 10 mg of beta-NADPH, and 0.1 mol/L Tris-HCL for 30 min at 37℃. The sections were then incubated in 0.1 mol/L PBS (pH 7.4) containing 0.2% Triton X-100 (1.0 mL) for 120 min, dehydrated in a graded series of alcohol, and sealed with neutral gum.

Under a light microscope, cells containing NADPH-

diaphorase, a marker for the presence of inducible nitric oxide synthase (NOS), were observed and the integral grey value was detected using an image analyzer.

Statistical analysis

Data were expressed as mean ± SD and analyzed with one-way ANOVA using SPSS11.0 software. $P < 0.05$ was considered statistically significant.

RESULTS

Effect of EA on ischemic ECG-ST

After occlusion of DAB, ECG-ST elevated significantly in model, PC6, SP4, PC6 + SP4 groups ($P < 0.05$, Figure 1A). Following AMI/R, ECG-ST elevated in the model group and declined in the three EA groups (PC6, SP4, PC6 + SP4). The ECG-ST values were significantly lower in PC6, SP4 and PC6 + SP4 groups than those in the model group at 10, 20 and 30 min after EA ($P < 0.05$). No significant difference was found among the three EA groups ($P > 0.05$). The ECG-ST values were still markedly higher in the SP4 and model groups than those in control group 30 min after EA ($P < 0.05$), and no significant difference was found between the PC6 and control groups or between the PC6 + SP4 and control groups.

Effect of EA on the amplitude of slow waves of EGG

After AMI, the slow wave amplitude of EGG declined remarkably ($P < 0.05$, Figure 1B), the slow wave amplitude of EGG in the model group decreased further, while those in the PC6, SP4 and PC6 + SP4 groups had an apparent recovery. The slow wave amplitude of the model group was significantly lower than that in the control group after AMI and AMI/R ($P < 0.05$). The slow wave amplitudes of the SP4, PC6 and PC6 + SP4 groups were comparable after AMI/R, and significantly higher in the SP4 group than that in the control group ($P < 0.05$). The effect of EA of SP4 was slightly stronger on increasing the slow wave amplitude of EGG.

Effect of EA on the frequency of slow waves of EGG

Following AMI, the frequency of slow waves of ischemic EGG decreased significantly in comparison with the basal values in each group ($P < 0.05$, Figure 1C). After AMI/R, the mean frequency of slow waves of ischemic EGG in the model group decreased further, and was still markedly lower than its basal value 30 min post-EA ($P < 0.05$). The mean frequencies of slow waves were significantly higher in the three EA groups than that in model group after AMI/R ($P < 0.05$). At 20 min after EA, the mean frequency of slow waves of ischemic EGG in the SP4 group was not significantly different from that in the control group ($P > 0.05$). At 10 min after AMI/R and EA, the mean frequencies of slow waves of ischemic EGG in the PC6 + SP4 and PC6 groups were obviously higher than that in the model group ($P < 0.05$, Figure 1C) and did not return to their basal values 30 min after EA

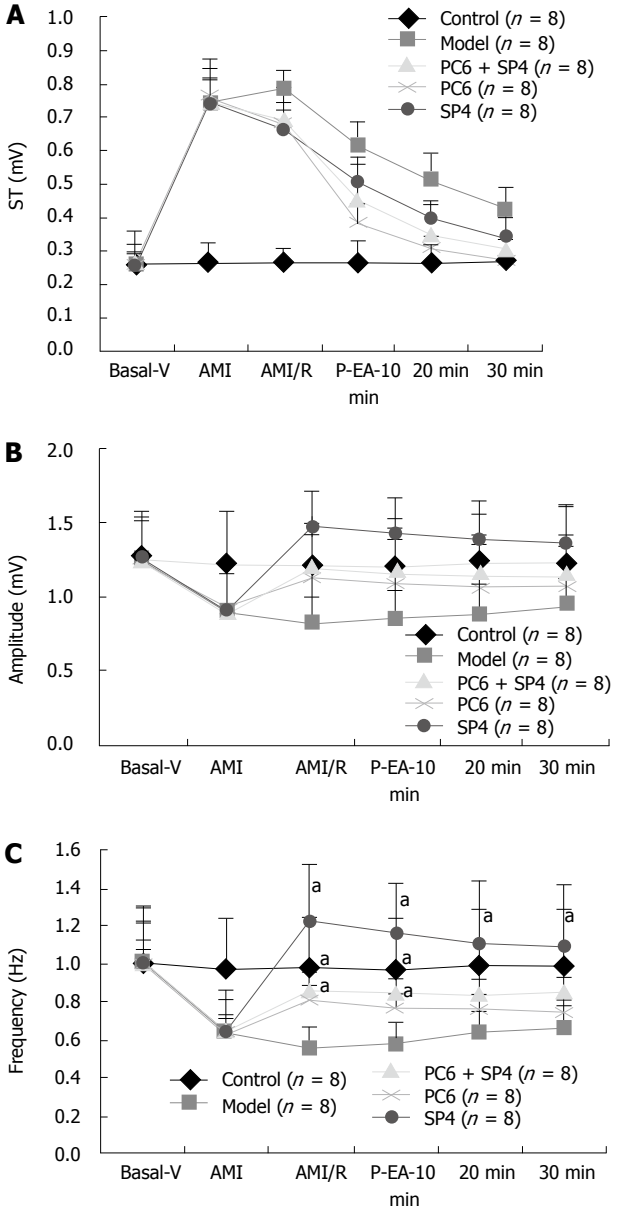

Figure 1 Changes in rabbit ECG-ST (mV, A), amplitude (mV, B) and frequency (pulses/s, C) of slow waves of electrogastrogram (EGG) in control, model, PC6, SP4 and PC6 + SP4 groups ($n = 8$ in each group) at different courses after acute myocardial ischemia and reperfusion (AMI/R). [a]$P < 0.05$ *vs* model group; Basal-V: Basal value; P-EA: Post-EA.

($P < 0.05$). The effect of EA of SP4 was slightly better on increasing the frequency of slow waves of ischemic EGG.

Effect of EA on the frequency of fast waves of ischemic EGG

During experiments, burst (fast) of waves ischemic of EGG appeared only in some animals, particularly after AMI/R. We randomly chose three rats in each group and analyzed the effect of EA (Table 1). The frequency of fast waves of ischemic EGG increased significantly from the beginning of AMI/R after EA in the SP4 group, while the frequencies of fast waves of ischemic EGG in the model, PC6 and PC6 + SP4 groups were close to that in the control group.

Table 1 Changes in mean frequency of EGG fast waves in rabbits at different time-courses after AMI/R and EA (mean ± SD, pulses/s, *n* = 3/group)

Groups	Basal values	AMI	AMI/R	P-EA 10 min	P-EA-20	P-EA-30 min
Control	1.90 ± 1.91	1.84 ± 1.82	1.89 ± 1.87	1.84 ± 1.82	1.90 ± 1.84	1.85 ± 1.83
Model	1.98 ± 0.93	0.91 ± 0.10	0.93 ± 0.91	1.05 ± 0.09	1.53 ± 0.61	1.76 ± 1.00
PC6	3.48 ± 1.52	1.92 ± 2.17	3.81 ± 3.12	3.47 ± 4.31	2.65 ± 2.83	2.10 ± 2.10
SP4	2.01 ± 0.38	1.26 ± 0.25	4.04 ± 1.19	3.89 ± 0.98	3.58 ± 0.93	2.93 ± 0.36
PC6 + SP4	1.45 ± 1.21	0.68 ± 0.85	1.61 ± 1.19	1.52 ± 1.18	1.30 ± 0.95	1.19 ± 0.90

Table 2 Mean integral grey values of NADPH-diaphorase /NOS expression in myocardium, gastric antrum and duodenum in rabbits (mean ± SD)

Groups	Cases	Myocardium	Gastric antrum	Duodenum
Control	4	122.92 ± 22.40	168.21 ± 58.30	127.01 ± 46.26
Model	4	212.93 ± 22.30	218.40 ± 23.30	190.71 ± 33.91
PC6	4	143.41 ± 38.01	171.11 ± 37.11	178.53 ± 40.05
SP4	4	191.63 ± 16.20	163.35 ± 24.85	140.86 ± 60.84
PC6 + SP4	4	184.72 ± 30.51	140.86 ± 60.84	178.09 ± 51.26

Effect of EA on the NOS activity of myocardium, gastric antrum and duodenum muscle

NADPH-diaphorase staining of myocardium, gastric antrum and duodenum (Figure 2A-C) showed that NADPH-diaphorase/NOS-expressed positive fibers, neurons and their processes had a purple/blue color, which was denser in the control group, moderate in the three EA groups and lightest in the model group.

In myocardium tissue (Figure 2A), NOS positive reaction product (in nerve fibers) presented a string-of-beads-like distribution (dark blue color) on the wall of larger blood vessels along the longitudinal axis of the smooth muscle fibers, and was denser in the control group, moderate in the PC6 group, milder in the PC6 + SP4 and SP4 groups, and lightest in the model group. In gastric antrum tissue (Figure 2B), most NOS-expressed neurons were round and oval in shape. Fewer irregular polygons and their cytoplasm were uniformly dark blue and their nuclei were relatively lighter in color. NOS-positive reaction product was denser in the control group, moderate in the SP4 group, milder in the PC6 and PC6 + SP4 groups, lightest in the model group. In the duodenum tissue (Figure 2C), the shape and color of NOS-expressed positive neurons in the control, model, PC6, PC6 + SP4 and SP4 groups were similar to those in the gastric antrum.

The integral grey values for myocardium, gastric antrum and duodenum increased in the model and PC6, PC6 + SP4 and SP4 groups compared with the control group after AMI/R (Table 2). Following EA, the integral grey values for myocardium and duodenum in the PC6 and SP4 group were closer to that in the control group, suggesting that EA could suppress AMI/R- induced down-regulation of NOS.

DISCUSSION

In the light of the theory of Chinese medicine, PC6 and SP4 are two convergent acupoints of the eight Meridians. PC6, the Luo-point of the Pericardium Meridian, is often used to treat palpitation, chest distress and thoracalgia in the upper energizer, gastralgia and vomiting of the middle energizer, and irregular menstruation of the lower energizer, *etc*. SP4, the Luo-point of the Spleen Meridian of Foot-Taiyin, is frequently employed to treat gastralgia, vomiting, abdominal pain, diarrhea, *etc*. These two acupoints, used in combination, can function well in relieving disorders of the heart and gastrointestinal tract, but related experimental investigations are fewer, in spite of being frequently used in clinic practice.

Results of the present study show that ECG-ST elevated significantly after occlusion of DAB, meaning occurrence of AMI. After release of the ligature (AMI/R), ECG-ST in the model group elevated further, while decreased significantly in the PC6, SP4 and PC6 + SP4 groups. At 20 min after EA in the PC6 group, and 30 min in the PC6 + SP4 group after EA, ECG-ST values returned to their basal levels before occlusion of DAB, and those in the SP4 and model groups were still higher than their respective basal levels. No significant differences were found in ECG-ST values at different time courses among the three EA groups, indicating that EA of PC6, SP4 and PC6 + SP4 can effectively promote the recovery of ischemic myocardial injury. Unfortunately, no synergistic action of EA of PC6 + SP4 on AMI was found in the present study. The results of EA of PC6 and PC6 + SP4 are similar to the reported findings[5,7,8,16].

After AMI, the cardiac blood-pumping function was weakened, leading to decrease in cardiac output and in perfusion volume in many organs, and disturbance of blood circulation, thus an abnormal change occurred in EGG.

The results of this study show that after AMI, the amplitude and frequency of slow waves of EGG decreased considerably, and decreased further in the model group, and did not return to their pre-AMI levels at 30 min after ligation of DAB, suggesting a remarkable suppression of the basal electrical activity of the gastric smooth muscle due to insufficient blood supply. In the three EA groups, both the amplitude and frequency of slow waves of EGG increased after EA stimulation. The amplitude of slow waves of EGG in the three EA groups returned to their pre-AMI basal levels at 30 min after EA. No significant difference was found in the amplitude of slow waves of EGG among the three EA groups. Twenty minutes after EA, the mean frequency in the SP4 group was not markedly different from that in the control group. Ten minutes

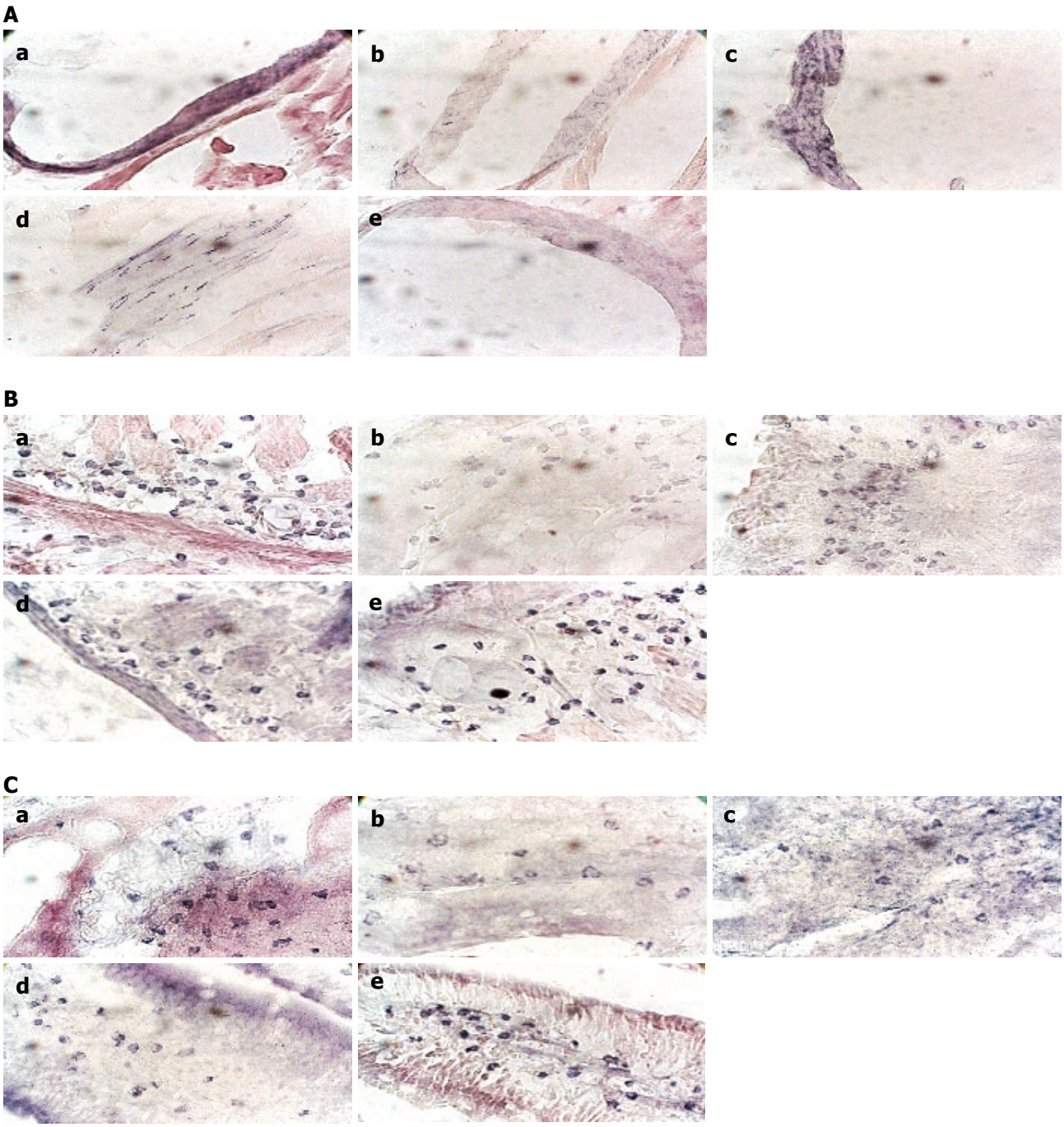

Figure 2 Photographs showing NOS expression in the myocardium of the rabbit heart base (left atrium, A), gastric antrum (B) and duodenum (C) in control (a), model (b), PC6 (c), PC6 + SP4 (d) and SP4 (e) groups. A: NOS positive reaction product (in nerve fibers) presented string-of-beads-like distribution (dark blue color) on the wall of larger blood vessels along the longitudinal axis of the smooth muscle fibers. NOS-positive product was denser in control group (a), moderate in PC6 group (c), milder in PC6 + SP4 (d) and SP4 (e) groups, lightest in model group (b); B: NOS positive reaction neurons distributing in the mucous layer and underlayer of the rabbit gastric antrum; C: NOS positive reaction neurons distributing in the mucous layer and underlayer of the rabbit duodenum tissues. Most of them were round and oval, and fewer irregular polygon, and their kytoplasm was uniformly dark blue and their nuclei were relatively lighter in color. NOS-positive reaction product was denser in control group (a), moderate in SP4 group (e), milder in PC6 (c) and PC6 + SP4 (d) groups, lightest in model group (b). (X 400).

after AMI/R and EA, the mean frequency in PC6 + SP4 and PC6 groups was obviously higher than that in the model group and did not return to their basal values 30 min after EA, indicating that the effect of EA of SP4 on the frequency of slow waves of EGG is stronger. It has been demonstrated that EA of PC6 and SP4 can ameliorate symptoms of abdominal pain, vomiting, *etc*, and the ratio of the main frequency of EGG between pre- and post-meal in functional dyspepsia patients[13,14,21,22].

It has been well-documented that the effect of acupuncture on regulating cardiac and gastrointestinal activities involves the nervous system, endocrine system, and multi-targets[1,4,12,14]. Improved microcirculation and appropriate amount of NO production may be one of the important factors. NO, a micromolecule gas, is

synthesized from *l*-arginine under the catalysis of NO synthase, and can activate guanylate cyclase of the cells and exert biological effects *via* the resultant cGMP. Continuous basal release of NO is very important for maintaining stable diastole state and base tension of the coronary artery and regulating blood pressure and myocardial blood perfusion[23]. Myocardial ischemia decreases myocardial NO concentration and NOS expression[24]. In our experiments, the blue positive reaction product in the myocardium, gastric antrum and duodenum was the densest in the control group, moderate in the three EA groups, and the lightest in the model group. The integral grey values for myocardium in the PC6 group, gastric duodenum in the PC6 + SP4 group were relatively denser, suggesting that NOS expression decreases apparently after AMI, while increases after EA, which may contribute to its effect on improving cardiac and gastric electrical activities in AMI/R rats. Our results are basically identical to other reported findings[25-29]. For example, Li *et al*[25] observed that EA of PC6 could up-regulate plasma NO and blood platelet α granule membrane protein-140 contents and reduce myocardial injury in myocardial ischemia rabbits. Luo *et al*[26] reported that acupuncture of PC6, SP4, *etc*, could relieve symptoms of peptic ulcer and elevate plasma NO level. In rats with alcohol-induced mucous membrane injury, EA of Zusanli (ST36) plus PC6 or SP4, or simple PC6, could effectively raise the contents of NO and epidemic growth factor in gastric mucosa tissue, and promote the synthesis and release of NO, thus protecting gastric mucosa from injury[27-29]. These results suggest that NO is able to mediate the effects of EA on improving cardiac and gastrointestinal functional activities. Our study did not show a synergistic action of EA of both PC6 and SP4 as reported[30].

In conclusion, EA of PC6 and SP4 can exert similar effects on cardiac and gastric activities. EA of one acupoint may simultaneously regulate the activities of multiple viscerae. Hence, in clinical practice, PC6 and SP4 used in combination are applicable to the treatment of both cardiac and gastric disorders.

COMMENTS

Background

Neiguan (PC6) and Gongsun (SP4), two convergent acupoints of the eight Meridians, are indicated for disorders of the stomach, heart and chest. In fact, the two acupoints are frequently used in combination to treat gastrointestinal disorders as nausea, vomiting, *etc*, in clinical practice. When used individually, PC6 mainly functions well in regulating problems of the heart, while SP4 is chiefly indicated for gastralgia, acute and chronic enteritis, vomiting, menstrual disorder, *etc*. The present study observed the effect of electroacupuncture (EA) of PC6 and/or SP4 on the activities of the heart and gastrointestine under pathological state (acute myocardial ischemia) from electrophysiological and histochemical aspects so as to analyze their action regularities.

Research frontiers

The correlation between acupoints and internal organs is a key theoretical basis of Chinese acupuncturology and acupuncture in clinical practice. The present study was undertaken to observe the relative specificity of EA of acupoints in regulating activities of the corresponding internal organs. In addition, joint application of two or more acupoints may have a synthetic effect or a counter action under certain circumstances. Since the related regularities and the underlying mechanism remain unknown, the present study observed the effects of EA of PC6 and /or SP4 on activities of the heart and gastrointestine simultaneously.

Innovations and breakthroughs

Fewer experimental studies have been conducted to observe the effect of EA of one or two acupoints on two internal organs at the same time. The present study demonstrated that EA of PC6, SP4 or PC6 + SP4 on myocardial ischemia-reperfusion (MI-R)-induced elevation of ECG-ST and decreased mean amplitude and frequency of slow waves of electrogastrogram (EGG), as well as reduced nitric oxide synthase (NOS) expression in the myocardium, gastric antrum and duodenum were all corrected significantly sooner or later. Moreover, EA of PC6 and SP4 could improve ECG-ST, and regulate NOS activity of myocardium and mucous membrane of duodenum.

Applications

The results of the present study provided certain new experimental evidence for treating disorders of the heart and gastrointestine by acupuncture of PC6 and SP4 and verified the theory of Chinese acupuncturology about the relatively specific effects of acupoints on their corresponding internal organs.

Terminology

According to Chinese medicine theory, there are 12 regular meridians distributed regularly in the human body, each meridian pertains to a certain Zang- or Fu-organ (internal organ). When a certain acupoint is stimulated with an acupuncture needle, the corresponding internal organ can be regulated effectively in functional activities by way of meridian, particularly under pathological state. However, the regulatied effect of the stimulated acupoint on the internal organ is definitely not absolute, some related organs may be influenced at the same time. Thus, when mentioning the acupoint specificity, it mainly refers to its relative specificity in function.

Peer review

The paper describes the characteristics, values and significance of electroacupuncture, as well as the effects of electroacupuncture on cardiac and gastric activities of rats with ischemia, which may be of some values for clinicians in their clinical practice.

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