Basic Investigations

Effects of Electroacupuncture at Different Points on Colorectal Distention-induced Changes in Blood Pressure, Electrogastrogram, Gastric Tension and Gastric Blood Flow

CHEN Shu-ping 陈淑萍, GAO Yong-hui 高永辉, WANG Jun-ying 王俊英, and LIU Jun-ling 刘俊岭

Objective: To investigate the specificity of the effects of electroacupuncture (EA) at different acupoints on gastric functional activity and gastric blood flow after colorectal distension (CRD) in the rat.

Methods: Fifty Wistar rats were randomly divided into 5 groups: a control group, a Zusanli group, a non-point group, a Taichong group and a Neiguan group. Rats were anesthetized after 18 h of fasting, and a rat model of nociceptive blood pressure elevation and abnormal electrogastrogram (EGG) and gastric tension (GT) was prepared by gasbag-induced CRD. EA was given bilaterally, and its effects on gastric blood flow (GBF) of the arcus vasculosi of the greater omentum, blood pressure, EGG fast wave properties, and gastric smooth muscle tension (GT) were quantified.

Results: CRD induced an increase in blood pressure that was significantly inhibited by EA at all points (all P<0.05), and the inhibitory effects were greater in the Zusanli and Neiguan groups compared to the non-point group (both P<0.05). CRD also caused reductions in GT and GBF, and in the amplitude, frequency and duration of EGG waves (all P<0.05). These effects were also reversed by EA. The effects of EA on EGG wave amplitude, GT, and GBF were superior in the Zusanli group compared to the Taichong group, Neiguan group, and the non-point group.

Conclusion: EA significantly counteracted CRD-induced changes in blood pressure, GBF, EGG, and GT. The effects of stimulation at Zusanli (ST 36) were significantly greater compared to other points, indicating relative specificity of this acupoint.

Keywords: electroacupuncture; Stomach Channel of Foot-Yangming; electrogastrogram; gastric motion; acupuncture effect; point specificity

It has been reported that acupuncture at Zusanli (ST 36), Neiguan (PC 6), and other acupoints can effectively alleviate epigastralgia, abdominal pain, and clinical symptoms and signs of irritable bowel syndrome (IBS).1-4 It can also inhibit colorectal distension (CRD)-induced hyperalgia of internal organs in rats with IBS, and colonic stimulation-induced chronic hypersensitivity of internal organs.5,6 Our previous research has shown that electroacupuncture (EA) at Zusanli (ST 36) or Neiguan (PC 6) inhibits the abnormal increase in mean arterial pressure caused by CRD, and regulates heart rate and the balance between sympathetic and vagal activity.7 These findings indicate that stimulation of the two acupoints can significantly alleviate pain in internal organs and beneficially regulate CRD-induced dysfunction of autonomic nerves. In addition, many previous studies have shown that EA can effectively regulate cardiovascular functions.8,9 The specificity of point action, in particular the specificity of points located at a same neural segment compared to points located at an adjacent neural segment, is still not understood. Most previous studies have tested the effects of stimulation of a single point on the functional activity of one organ, whereas less attention has been given to regulatory effects of multi-point stimulation on one organ, or one-point stimulation on multiple organs. Therefore, we studied the effects of EA at Zusanli (ST 36), Neiguan (PC 6), Taichong (LV 3), or a non-point, on blood pressure (BP), electrogastrogram (EGG), gastric tension (GT) and blood flow in rats with a CRD-induced nociceptive increase in blood pressure (BP), and analyzed the action of different points on the activities of the two systems.

MATERIALS AND METHODS

Experimental Animals and Grouping
Fifty healthy adult Wistar rats, body mass 200–250 g, were purchased from the Animal Center, Chinese Academy of Medical Sciences (License No: SCXX-Army 2007–004). Animals were randomly divided into five groups: 1) control group, 2) EA Zusanli group, 3) EA non-point group, 4) EA Taichong group, and 5) EA Neiguan group. Before the experiment, the rats had free access to water and fasted for 18–24 h. All animal experiments followed the ethical regulations in China Academy of Chinese Medical Sciences, Beijing 100700, China

Correspondence To: LIU Jun-ling, E-mail: jlliu2765@yahoo.com.cn

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**Main Experimental Instruments**
PowerLab multi-functional biosignal collection and analysis system (ADInstruments, Sydney, Australia), Biopac multi-channel biosignal collection system (BIOPAC Systems, Inc., Goleta, CA, USA), TSD104A blood pressure transducer, TSD105A tension transducer, DA100C universal amplifier, ECG100C EGG amplifier, LDF100C laser Doppler flowmeter, DRB-2D electrothermic Bianshì temperature control apparatus, and HANS’s electroacupuncture treatment instrument (LH202H, Nanjing, China).

**Preparation of Internal Organ Pain Model**
The rats were anesthetized by intraperitoneal injection (0.4 mL/100 g body weight) of mixed solution (1:2) of 25% urethane (Beijing Chemical Reagents Company, Beijing, China) and 1.5% chloralose (Sigma-Aldrich Co., St. Louis, MO, USA). One end of a polyethylene catheter was covered with a gasbag of 2.5 cm in diameter, with the air in the catheter and the gasbag previously evacuated. Then, the gasbag was smeared with paraffin and inserted about 3–4 cm into the anus, and fixed with adhesive plaster to the tail-root. The catheter connected with the gasbag was linked to a TSD104A pressure transducer and a Biopac DA100C amplifier. The gasbag was slowly filled with water to increase the pressure, which was controlled at about 50–60 mmHg for CRD, for 5 min. When the blood pressure was raised by 15%–20%, the model was regarded as successful.

**Recording of Blood Pressure**
During anesthesia, a catheter filled with 0.2% heparin was inserted about 10 mm into the proximal end of the middle segment of the common carotid artery. The catheter was linked to a pressure transducer via a triton instrument and an injector, and the arterial pressure signal was amplified, and recorded and stored with a computer sample collection and analysis system. The body temperature of the rat was kept at 37±1°C.

**Recording and Analysis of EGG and Gastric Tension**
The anesthetized rat was fixed on an experimental table in the supine position. A medial incision was made in the abdominal wall below the xiphioid process, and a surgical thread was penetrated through the blood vessel-free area at the middle part of the stomach body, near the lesser curvature of the stomach. A leading thread was fixed on the rat table and another leading thread was sutured in the stomach near the gastric antrum, and fixed to the Biopac-TSD105A tension transducer. The interval between the two leading threads was 1–1.2 cm, and the initial tension was set to 0.45 g each session. The gastric tension signal was fed to an ECG100C amplifier via the transducer, and then displayed and stored on the computer. Determination of EGG: A bipolar stainless-steel electrode was fixed in the subserous layer of the stomach near the gastric antrum between the two threads with an interval of about 6–8 mm between the two electrodes. Paraffin was added to the exposed part of the stomach, and the wound was covered with a polyethylene membrane to maintain body temperature and prevent drying. A polyethylene spacer (1.5×1 cm) was placed under the electrode to prevent short-circuit (Figure 1). The EGG signal was amplified and recorded to computer.

After stabilization of the recording, a 2 h recording was obtained. Because the number of electric discharges in each cluster showed large differences between animals, the fast wave cluster number, amplitude (μV), and duration (ms) were calculated each minute, and the mean tension of gastric smooth muscle was simultaneously recorded and analyzed.

**Detection of Volume of Blood Flow in Stomach**
Under anesthesia, the rat abdominal cavity was opened with a transversal cut from the interior margin, and the stomach was exposed. A LDF100C laser Doppler flowmeter was used, and a TSD145 laser Doppler probe needle was fixed, slightly touching, to the greater curvature of stomach near the arcus vasculosi of the greater omentum (Figure 1). The position was relatively fixed throughout each session. After stabilization of the value, it was recorded, and the signals in the flowmeter were changed into blood perfusion units (BPU). Recording of the curve output was carried out with AcqKnowledge 3.8.1 software.

**Point Selection and Parameters of EA**
The points were located according to Hu Xing-ban’s “Points Atlas of Rats”.10 Zusanli (ST 36) was located about 5 mm lateral to the apex of the fibular head on the bilateral hind limbs, and was perpendicularly punctured to a depth of 5–6 mm. The non-point was selected as the point about 1.0 cm lateral to Zusanli (ST 36), and it was
perpendicularly punctured to a depth of 5–6 mm. Taichong (LR 3) was located on the back of the feet, in the excavation behind the space of the first metatarsal bone, and it was perpendicularly punctured to a depth of 3–4 mm. Neiguan (PC 6) was located 3 mm from the wrist joint, between the ulna and radius on both sides, and it was perpendicularly punctured to about 3 mm.

Parameters of EA: Frequency 2/15 Hz, intensity 1–2 mA, duration 15 min. Before CRD, two acupuncture needles of 0.5 \text{ cun} were inserted into the above points bilaterally, and the needles were connected with the acupuncture apparatus. Acupuncture stimulation was given after 2 min of dilation of the anus.

Statistical Analysis
Data were expressed as mean ± standard deviation (SD). One-way ANOVA was used for analysis of differences among the groups, and the least significant difference (LSD) test was used for paired comparisons of means between groups. \( P<0.05 \) was regarded as statistically significant.

RESULTS

Effects of EA on CRD-induced Changes in Blood Pressure
After CRD, the blood pressure was increased by 15%–20% in all groups, but was significantly lower in all the EA groups compared to the control group \(( P<0.05 \)) . The blood pressure was significantly lower in the Zusanli and Neiguan groups after 5 min of EA, and in the Zusanli group after 15 min of EA, compared to control and non-point groups \(( P<0.05 \) ) . The effect of acupuncture at Zusanli (ST 36) lasted for 10 min after ceasing EA. These findings indicate that acupuncture at Zusanli (ST 36), the non-point, Taichong (LR 3) and Neiguan (PC 6) significantly inhibits the CRD-induced increase in blood pressure. This effect was most obvious for acupuncture at Zusanli (ST 36), followed by Neiguan (PC 6) (Figure 2).

Effects of EA on Fast Wave Amplitude of EGG after CRD
Fasted rats had more frequent break-outs of fast wave EGG activity, mostly with cluster-form, but often also showing bi-directional asymmetric needle-form waves. After CRD, the blood pressure increased and the amplitude of the fast wave EGG activity was significantly decreased in all groups (Figure 3). In some cases, the fast wave activity was completely inhibited. This decrease in the fast wave amplitude was rapidly reversed in all of the EA groups, with significant increases in the Zusanli group compared with the control group \(( P<0.05 \) ). After 5 min of EA, the wave amplitude was significantly higher in the Zusanli group than in the other groups \(( P<0.05 \) ). After 15 min of EA, the amplitude was still significantly higher compared to the control and Taichong groups (both \( P<0.05 \) ). The effect of EA in the Zusanli group lasted for 40 min after ceasing EA.

Effects of EA on Fast Wave Frequency of EGG after CRD
Fast wave frequency of EGG appeared at about 2 clusters per min in the normal fasted rat, with large individual difference in the number of electric discharges per cluster (from tens to hundreds). Therefore, the cluster number per minute was calculated. After CRD, the number of fast wave clusters was significantly decreased in all groups (Figure 4). At 5 min of EA, the fast wave frequency in the Zusanli group was significantly higher than in the control group \(( P<0.05 \) ). There were no significant differences in fast wave frequency at the other time points \(( P>0.05 \) ) between the groups.

Figure 2. Effects of EA on CRD-induced changes in blood pressure (mean ± SD, mmHg, 10 rats/group). \( \Delta : P<0.05 \) compared with the control group; \( \bullet : P<0.05 \) compared with the non-point group.

Figure 3. Effects of EA on fast wave amplitude of EGG after CRD (mean ± SD, \( \mu \text{V} \), 10 rats/group). \( \Delta : P<0.05 \) compared with the control group; \( \bullet : P<0.05 \) compared with the non-point group; \( \bullet \bullet : P<0.05 \) compared with the Taichong group; \( \bullet \bullet \bullet : P<0.05 \) compared with the Neiguan group.

Figure 4. Effects of EA on Fast Wave Frequency of EGG after CRD (mean ± SD, cluster/min, 10 rats/group). \( \Delta : P<0.05 \) compared with the control group.
Effects of EA on Fast Wave Duration of EGG after CRD

After CRD, the duration of each EGG fast wave cluster was shortened in all the groups (Figure 5), and it was significantly prolonged by EA at Zusanli (ST 36) \((P<0.05)\). This effect lasted for 10 min after ceasing EA. In the other EA groups, there was a trend towards a similar improvement but these differences were not statistically significant \((P>0.05)\).

![Figure 5. Effects of EA on Fast Wave Duration of EGG after CRD (mean ± SD, ms, 10 rats/group). ▲: \(P<0.05\) compared with the control group.](image)

Effects of EA on Tension of Gastric Smooth Muscle after CRD

The gastric motion conformed to the Activity rules of EGG cluster-form fast wave in the normal rat. When the cluster-form fast wave was generated, the tension of the gastric muscle increased, and then mechanical contraction of the stomach appeared. After CRD, the fast wave of the stomach was inhibited and GT decreased (Figure 6). After EA, GT increased in all the EA groups, with the most obvious increase in the Zusanli group (Figure 2). After 15 min of EA, GT was significantly higher than in the control group, the non-point group and the Taichong group \((P<0.05)\), and the effect lasted for 10 min after ceasing EA.

![Figure 6. Effects of EA on Tension of Smooth Muscle of the Stomach After CRD (g, mean ± SD, 10 rats/group). ▲: \(P<0.05\) compared with the control group. ▼: \(P<0.05\) compared with the non-point group; ▣: \(P<0.05\) compared with the Taichong group.](image)

Effects of EA on Blood Flow in the Stomach after CRD

The volume of blood flow in the arterial arc of the greater omentum of the stomach was significantly decreased in all the groups after CRD. Compared with the control group, the blood flow increased significantly in the Zusanli group \((P<0.05)\), but there were no significant effects in the other EA groups. Ten minutes after ceasing EA, the blood flow perfusion was still significantly increased in the Zusanli group compared to the other groups \((P<0.05)\). Thirty minutes after ceasing EA, the blood flow was gradually restored. The best restoration of blood flow was seen in the Zusanli group, with an almost complete return to normal levels.

![Figure 7. Effects of EA on Volume of Blood flow in the Stomach after CRD (mean ± SD, BPU, 10 rats/group). ▲: \(P<0.05\) compared with the control group; ▼: \(P<0.05\) compared with the other groups; ▣: \(P<0.05\) compared with the Neiguan group.](image)

Analysis of the Correlation between the Volume of Blood Flow in the Serous Membrane of the Stomach and the Volume of Blood Flow in the Arterial Arc of the Greater Omentum

During the experiment, the blood flow in the arterial arc of the greater omentum was stable, averaging 1045–1138 BPU. Blood flow data were used for linear correlation analysis, with blood flow in the arterial arc of the gastric greater omentum in normal and CRD rats as the independent variable and blood flow in the gastric serous membrane in normal and CRD rats as the dependent variable. The blood flow in the arterial arc of the greater omentum was positively correlated with the blood flow in the gastric serous membrane \((r=0.846, P<0.01)\) (Figure 8).

![Figure 8.](image)
and abnormal defecation in patients with IBS. Experiments in animals have confirmed that EA at Zusanli (ST 36) and other points can alleviate acute inflammatory pain of internal organs, and inhibit CRD-induced hyperalgia of internal organs in rats with IBS or colonic stimulation-induced chronic hyperactivity of internal organs. CRD-induced pain can stimulate sympathetic activity, and thereby increase blood pressure. Therefore, the decrease in blood pressure caused by EA may be mediated by inhibition of pain in internal organs, causing regulation of autonomic nerve activity.

Apart from inducing significant changes in blood pressure, CRD also can inhibit electric and mechanical activity of the stomach and intestine in humans and animals. This was observed in this study. In addition to the elevation in blood pressure, the amplitude of EGG fast waves decreased, the frequency of cluster-form waves was reduced, the cluster duration shortened, and the tension of gastric smooth muscle decreased. After EA at Zusanli (ST 36), the decrease in the amplitude of fast waves was smaller, and the duration was increased compared with the control group. Further, the frequency of cluster-form wave activity and the gastric tension were significantly increased. The inhibition of the decrease in fast wave amplitude was significantly stronger compared to the non-point group, the Taichong group and the Neiguan group. The increased gastric tension was significantly superior to that in the Taichong group and the non-point group. These results further indicate that the Zusanli (ST 36) shows an acupoint-action relative specificity, and is closely correlated with gastric activity. Studies on the specificity of acupoint action have been somewhat conflicting, but in general, the conclusions have been similar. For example, Zhao, et al. found that EA at Sibai (ST 2) or Dicang (ST 4) has an exciting effect on the amplitude, duration and peak cluster number in EGG recordings, with the effect of Sibai (ST 2) stimulation being the strongest, followed by stimulation of Dicang (ST 4). EA at Quanliao (SI 18), a point lateral to Sibai (ST 2), however, does not have any significant effect on electric activity of the stomach. In the rabbit model of gastric mucosa injury induced by gastric perfusion of alcohol, acupuncture at Sibai (ST 2), Zusanli (ST 36) and Liangmen (ST 21) can all improve the inhibited gastric motion, with stimulation of Sibai (ST 2) showing the strongest effects. In addition, acupuncture at Zusanli (ST 36) had a significant regulative action on gastric hyperfunction induced by stimulation of the lateral hypothalamic area in the rabbit.

Blood flow of the stomach plays a supportive role in gastric function and is an important index reflecting gastric function. At present, there are many methods of determining tissue blood flow. Among them, laser Doppler flowmetry is a non-invasive, convenient, rapid, and sensitive method, and it has been used for the study of the hemodynamics of microcirculation in many organs.
However, most animal experiments measure blood flow of the gastric mucosa and other regions by placing a probe directly on the tissue through the stomach incision. It has been confirmed that the blood flow volume in the serous coat in the gastric tissue is linearly correlated with blood flow volume in the mucosa.20,30 Because the range of the probe is limited (1 mm³), however, pressure changes induced by the probe pressing to the tissue surface (ischemia), causing mechanical stimulation (congestion), or, worse, sticking to the tissue, will cause changes in the blood flow volume in the tissue, influencing stability and producing measurement errors.31 Most of the blood in the gastrointestinal tract can reflux into the portal system. The greater omentum is the thin and fat-rich abdominal membrane linking the greater curvature of stomach and the transverse colon.32 The greater omentum has a rich vascular net, and contraction and dilation of these blood vessels have a regulative action on gastrointestinal blood flow and portal pressure.33 We found that the greater omentum could attain stable blood flow, and demonstrated that blood flow volume in the greater omentum was positively correlated with that in the serous coat of the stomach. After CRD, the blood flow volume in the arterial arc of the greater omentum decreased significantly, and it was increased during EA and after 5 and 10 min after EA, indicating that EA at Zusanli (ST 36) and other point areas improve gastric EGG activity, gastric tension, and other functions, by improving microcirculation. It has been shown that stress significantly decreases gastric blood flow volume in the rat,34 whereas acupuncture at Zusanli (ST 36) or Yanglingquan (GB 34) can significantly increase blood flow in the gastric mucosa in the stressed rat.35 Consistent with our results, acupuncture at Zusanli (ST 36) not only increased perfusion of the middle and small blood vessels in the stomach, but also to varying degrees increased the blood flow volume of microcirculation in other areas of the stomach, with more obvious increases in the parts near the middle and small blood vessels.36

In summary, we studied the ability of acupuncture to counteract the increase in blood pressure and inhibition of EGG and gastric motion caused by CRD-induced pain in internal organs. The results indicate that acupuncture at the Stomach Channel of Foot-Yangming specifically regulates EGG activity, gastric motion, gastric blood flow, and blood pressure.

REFERENCES


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