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ORIGINAL PAPER

Acupuncture at Both ST25 and ST37 Improves the Pain Threshold of Chronic Visceral Hypersensitivity Rats

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Abstract Previous studies demonstrated the efficacy of electro-acupuncture (EA) in relieving chronic visceral hypersensitivity (CVH) in IBS rats. However, ST25 which is a key acupoint for patients with IBS has not been reported in these experiments. Eight CVH rats were treated by EA at both ST25 and ST37 for 20 min, once daily for seven consecutive days, model rats ($n = 8$) and normal rats ($n = 8$) as controls. After the first EA treatment, the abdominal withdrawal reflex scores were investigated to evaluate the pain threshold. After seven EA treatments, the concentrations of 5-hydroxytryptamine (5-HT), 5-HT3 receptor (5-HT3R) and 5-HT4 receptor (5-HT4R) in colon tissue were assayed quantitatively by enzyme-linked immunosorbent assay. The results showed that EA improved the pain threshold of CVH rats, reduced the 5-HT concentration and increased the 5-HT4R concentration, but had no effect on the 5-HT3R concentration. Further studies are needed to optimize the choice of two-matching points for EA in the treatment of CVH rats.

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Introduction

Irritable bowel syndrome (IBS) is a functional bowel disorder characterized by chronic and recurrent hypogastralgia and changes in bowel evacuation habits, without specific pathological changes [1]. Nowadays, acupuncture is a focus of IBS treatment, although four acupuncture trials demonstrated no efficacy or minimal superiority over placebo/control in treatment of patients with IBS [2–5]. Further studies have demonstrated the effects of acupuncture on patients with IBS. Schneider et al. [6] found that pain relief in patients with IBS was associated positively with increased parasympathetic tone in acupuncture group, but not in sham acupuncture group. Xiao and Liu [7] reported that acupoint transcutaneous electric nerve stimulation was effective in reducing rectal hypersensitivity in patients with diarrhea-predominant IBS. Liu et al. [8] and Chan et al. [9] also reported acupuncture was an effective modality for the treatment of IBS. Our previous studies also indicated that acupuncture was a potentially valuable therapeutic remedy for patients with IBS [10, 11].

Other studies [12–14] demonstrated the efficacy of electro-acupuncture (EA) in relieving chronic visceral hypersensitivity (CVH) in postnatal development of rats [15]. The chosen acupoints, however, varied in those studies as shown in Table 1. Acupoint ST25 has not been reported in these experiments. ST25 and ST37 are two key acupoints chosen in this study based on our clinical treatments of patients with IBS since the 1980s [10, 11]. Moreover, our previous experiment showed that EA at

Table 1 Acupoints chosen in CVH rats

Acupoints	Animal model	Decreased visceral hypersensitivity (mmHg)	Reference
ST36, ST37	Neonatal rats	20, 40, and 60	[12]
ST36, SP6	Neonatal rats	40, 60 and 80	[13]
ST36, ST37	Neonatal rats	20, 40, 60, and 80	[14]

ST25 could increase the threshold pressure level in IBS model rats, which were set up with stress of binding limbs and colorectal distention [16]. Thus, we focused here on whether EA at both ST25 and ST37 could decrease CVH in postnatal development of rats.

5-Hydroxytryptamine (5-HT) plays key roles in intestinal peristalsis and secretion, and in sensory signaling [17]. It activates intrinsic and extrinsic primary afferent neurons to initiate, respectively, peristaltic and secretory reflexes, and to transmit information to the central nervous system [17]. It is mainly released from enterochromaffin cells and is regulated by the 5-HT3 receptor (5-HT3R), and the 5-HT4 receptor (5-HT4R), which induce visceral pain in the enteric nervous system [18]. Tian et al. [13] reported that EA could regulate the expression of 5-HT and 5-HT4R, assayed semi-quantitatively by immunohistochemistry. Therefore, we also investigated the effect of EA at both ST25 and ST37 on 5-HT, 5-HT3R and 5-HT4R, assayed quantitatively by enzyme-linked immunosorbent assay (ELISA).

Experimental Procedure

Animals

Twenty-four neonatal male Sprague–Dawley (SD) rats (5 days old) were obtained from the Experimental Animal Center of Shanghai University of Traditional Chinese Medicine. Eight rats were housed with a nursing adult female rat in a cage at room temperature (22 ± 2°C) and 60 ± 5% humidity. After 3 days, the 24 rats were assigned randomly to one of three groups: the normal group ($n = 8$), the model group ($n = 8$), and the EA group ($n = 8$). All rats in the study were used strictly in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Model Establishment

The CVH model was established according to Al-Chaer et al. [15]. Colorectal balloon distention (CRD) was performed to assess the abdominal withdrawal reflex (AWR) as a surrogate marker of visceral pain in the conscious neonatal SD rats 8 days after their birth. A lactoprene balloon (length 20.0 mm, diameter 2.0 mm) with 0.2 ml of air was inserted slowly into the descending colon for 1 min twice a day, with a 30 min interval to minimize any pain or discomfort, continuously for 7 days.

Location of ST25

The precise location of the abdominal points is defined by dividing the distance between the symphysis pubis and the basis of the xyphoid process of the sternum into 13 sections. Each section is defined as a 'cun' according to traditional Chinese medicine. Accordingly, ST25 is located on a horizontal line five 'cun' above the symphysis pubis and two 'cun' lateral to the midline.

EA Treatment

After the establishment of the CVH rat model, EA was applied with needles (0.25 mm in diameter) inserted bilaterally to a depth of approximately 0.5 cm at Tianshu (ST25) and Shangjuxu (ST37) (5 mm lateral to the anterior tubercle of the tibia and 20 mm below the knee joint), with a square wave (alternately at 100 Hz for 3 s and 2 Hz for 3 s, amplitude 0.2–0.6 ms, intensity 1 mA) for 20 min, once a day for seven consecutive days. The rats of the normal and model groups were not treated with EA, and the fixation of rats was the same as that of the EA group rats.

Abdominal Withdrawal Reflex Scores

At the first EA treatment, the AWR scores were investigated to evaluate the pain threshold for CVH in the IBS rats. The AWR scores were assigned according to the scale of Al-Chaer et al. [15]. When the balloon was inserted into the descending colon, CRD was produced by rapidly inflating the balloon at strengths of 20, 40, 60, and 80 mmHg for a period of 20 s. Each score was tested three times, and each rat was tested by two different persons who were not involved in this research project. There were 3 min intervals between the two tests, to allow the rats to adapt.

Concentrations of 5-HT, 5-HT3R and 5-HT4R

After seven EA treatments, segments of distal colon (5 cm in length) were removed from the rats and homogenized in phosphate buffered saline (10%, ratio of colon weight and PBS volume), and centrifuged at 4,000*g* at 4°C for 30 min. The 5-HT, 5-HT3R and 5-HT4R content of supernatant were assayed by ELISA. ELISA kits were used strictly according to the manufacturer's instructions (Bionewtrans Pharmaceutical. Biotechnology Co. Ltd. Franklin, MA.), with the results expressed per wet weight of tissue samples (pg/ml).

Data Analysis

The SPSS 10.0 statistical package (SPSS Inc., USA) was used and data were analyzed by ANOVA or unpaired *t*-test, as applicable. When appropriate, post-hoc tests were assessed using the LSD test if equal variances were assumed or with Dunnett's T3 test if equal variances were not assumed. Differences with $P < 0.05$ were considered significant.

Results

Effects of a Single EA Treatment on AWR Scores in IBS Rats

As shown in Fig. 1, the AWR scores in response to graded CRD (20, 40, 60 and 80 mmHg) were 0, 1.1 ± 0.2, 2.9 ± 0.2, 3.0 ± 0.2 in the normal group, and 1.9 ± 0.2, 3.1 ± 0.6, 3.5 ± 0.4, 4.0 ± 0.1 in the model group, and 0.2 ± 0.2, 1.3 ± 0.3, 3.0 ± 0.0, 3.5 ± 0.4 in the EA group, respectively. Thus the AWR scores for the IBS model rats were higher than those for the normal rats, and were lower than those for the model rats after EA treatment. This indicated that the EA treatment had a beneficial effect on the pain thresholds of the IBS rats.

Effects of EA on the 5-HT Concentrations in the Colon Tissue of IBS Rats

As shown in Fig. 2, the 5-HT concentrations in the colon tissue were 66.4 ± 10.4 pg/ml in the normal group, 82.1 ± 14.8 pg/ml in the model group and 64.5 ± 13.2 pg/ml in the EA group, respectively. These data indicate the 5-HT concentrations in the colon tissue in IBS rats were significantly increased after CRD stimulation, and were reduced after EA treatment. The results indicated that EA could reduce the concentration of 5-HT in the colon tissue of CVH rats.

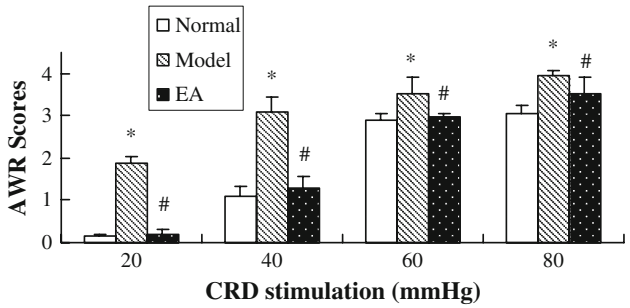

Fig. 1 Effects of a single EA treatment on the AWR scores of IBS rats. The AWR scores in response to CRD in model group were significantly higher than in normal group, and those in the EA group were significantly lower after a single EA treatment. Data are expressed as mean ± SD. * $P < 0.05$ vs. normal, # $P < 0.05$ vs. model

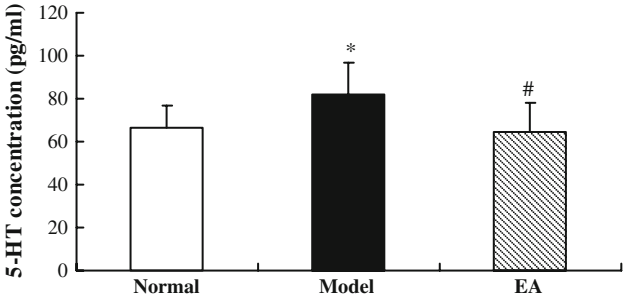

Fig. 2 Effects of EA on the 5-HT concentration in colon tissue. The 5-HT concentrations in colon tissue in the model group were significantly higher than that in the normal group, and those were significantly lower after EA treatment. Data are expressed as mean ± SD. * $P < 0.05$ vs. normal, # $P < 0.01$ vs. model

Effects of EA on the 5-HT3R Concentrations in the Colon Tissue of IBS Rats

The 5-HT3R concentration in the model group (40.6 ± 6.7 pg/ml) was not statistically different from that in the normal group (42.7 ± 5.2 pg/ml), and EA (44.0 ± 12.5 pg/ml) had no effect on the 5-HT3R concentration compared to that in the untreated model group (Fig. 3). This demonstrated that 5-HT3R might not be involved in the regulation of the visceral pain threshold in CVH rats.

Effects of EA on the 5-HT4R Concentrations in the Colon Tissue of IBS Rats

The 5-HT4R concentration in the model group (95.6 ± 15.4 pg/ml) was statistically different from that of the normal group (125.0 ± 12.7 pg/ml), and EA (113.5 ± 12.7 pg/ml) increased the 5-HT4R concentration compared to that in the untreated model rats (Fig. 4). This

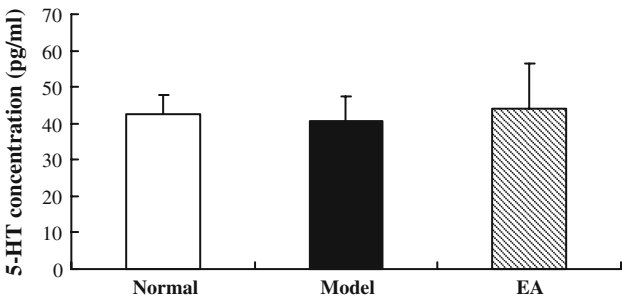

Fig. 3 Effects of EA on the 5-HT3R concentrations in colon tissue. There are no significant differences among the three groups. Data are expressed as mean ± SD

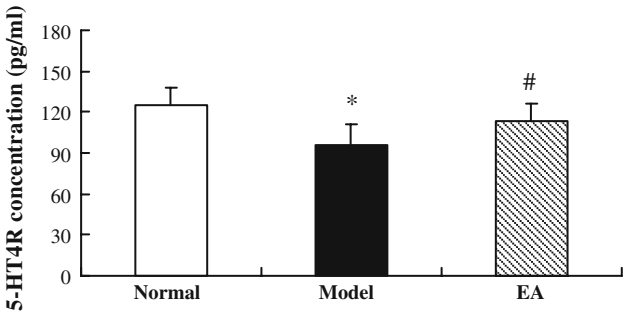

Fig. 4 Effects of EA on the 5-HT4R concentrations in colon tissue. The 5-HT4R concentrations in colon tissue in model group were significantly lower than that in the normal group, and those were significantly higher after EA treatment. Data are expressed as mean ± SD. * $P < 0.01$ vs. normal, # $P < 0.05$ vs. model

observation indicated that EA could reduce the 5-HT4R concentration in CVH rats.

Discussion

Effect of a Single EA Treatment on AWR Scores in IBS Rats

The IBS model was established by applying continuous colonic stimulation to SD rats from the neonatal period to adulthood, and visceral hypersensitivity was consistent with the pathological appearance of IBS. As shown in Fig. 1, the AWR scores for the IBS model rats were higher than those for the normal rats, and were lower than those for the model rats after EA treatment. This indicates that the EA treatment has a beneficial effect on the pain thresholds of the IBS rats. This is similar to previous studies [12–14].

However, there are different effects in applying EA with different two-matching points in different experiments as shown in Table 1. EA with two-matching points ST36 + ST37 decreased visceral hypersensitivity at 20, 40, and 60 mmHg [12]. EA with two-matching points ST36 + SP6 decreased visceral hypersensitivity at 40, 60, and 80 mmHg [13]. EA with the two-matching points ST36 + ST37 decreased visceral hypersensitivity at 20, 40, 60, and 80 mmHg [14]. In this study, EA with two-matching points ST25 + ST37 also decreased visceral hypersensitivity at 20, 40, 60, and 80 mmHg. This indicates the points used may vary among individuals.

In traditional Chinese medicine, ST25 is a 'mu' point of the large intestine, ST37 is a 'Xiahe' point of the large intestine, ST36 is a 'Xiahe' and a 'He' point of Stomach Meridian of Foot-Yangming. The three points are chief points used in treating patients with IBS. Previous studies have shown the efficacy of EA on CVH rats using two-matching points such as ST36 + ST37 and ST36 + SP6. Two-matching points ST25 + ST37 are also effective in this study. In addition, the points such as Dachangshu (BL25), Zhongwan (RN12) and Qihai (RN6) are usually chosen. There should be an optimal choice of two-matching points amongst those points. The optimal choice of two-matching points could be the best representative effect of EA in CVH rats. So the optimal choice of two-matching points should be established in future studies on the mechanisms of EA action on CVH rats.

Effects of EA on the 5-HT Concentrations in the Colon Tissue of IBS Rats

5-HT is an important signaling molecule and induces visceral pain in the enteric nervous system [18]. Using semiquantitative immunohistochemistry, Tian et al. [13] found that CVH rats had elevated 5-HT concentrations in colon mucosa. In our study, quantitative ELISA data also showed that 5-HT concentrations in the colon in CVH rats are significantly higher than that in normal rats. These studies show that close relationship between elevated 5-HT concentrations in colon mucosa and CVH. In addition, the 5-HT concentrations abnormally increased in the colon tissue of CVH rats can be reduced by EA treatment [13]. This observation was confirmed in our study, as shown in Fig. 2. These results demonstrate that EA could reduce the 5-HT concentrations in colon tissue of CVH rats.

Effects of EA on the Concentrations of 5-HT3R and 5-HT4R in the Colon Tissue of IBS Rats

In the gastrointestinal tract, the primary receptors of 5-HT appear to be 5-HT3R and 5-HT4R, which are involved in the regulation of 5-HT release from enterochromaffin cells after mucosal stimulation [19, 20]. 5-HT then diffuses to the nerve endings and stimulates peristalsis by binding to 5-HT3R and 5-HT4R located on the enteric nerves [21]. Visceral pain was relieved by blocking 5-HT3R and 5-HT4R [22, 23], and 5-HT4R agonists can reduce the rectal sensation thresholds of rats and humans [24–26]. These observations suggest that 5-HT and its receptors are involved in the modulation of visceral sensitivity and perception.

The concentrations of 5-HT3R in the model rats were not statistically different from that of normal rats (Fig. 3), corroborating the results of others [13]. Furthermore, EA increased the concentration of 5-HT4R compared to that in the untreated model rats (Fig. 4). These demonstrate that EA can reduce the concentration of 5-HT by activating 5-HT4R, whereas 5-HT3R may not be involved in the regulation of the visceral pain threshold in CVH rats. However, in order to identify whether 5-HT4R is involved in the regulation of the visceral pain threshold in CVH rats

by EA, 5-HT4R antagonists such as GR-113808 should be used in further studies.

In conclusion, this study has demonstrated that EA at both ST25 and ST37 has a beneficial effect on increasing the pain thresholds of the IBS rats. The optimal choice of two-matching points for EA in treatment of CVH rats should be studied further. EA at both ST25 and ST37 relieves chronic visceral hyperalgesia via serotonin pathway in the colon tissue. 5-HT4R may be involved in the regulation of the visceral pain threshold in CVH rats.

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