Basic Study

Comparison of the analgesic effects between electro-acupuncture and moxibustion with visceral hypersensitivity rats in irritable bowel syndrome

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Author contributions: Zhao JM, Li L and Chen L contributed equally to this work; Shi Y and Wu HG designed the research; Zhao JM, Li L and Chen L performed the research; Li YW, Shang HX and Wu LY analyzed the data; Zhao JM, Li L and Chen L wrote the paper; Weng ZJ and Bao CH made critical revisions to this manuscript.

Supported by National Basic Research Program of China (973 Program), No. 2009CB522900 and No. 2015CB554501; and National Natural Science Foundation of China, No. 30973784.

Institutional review board statement: The study was review and approved by the Ethics Committee of Yueyang Hospital of Integrated Traditional Chinese and Western Medicine, Shanghai University of Traditional Chinese Medicine.

Institutional animal care and use committee statement: All animals used in this study were treated according to the “Beijing Administration Rule of Laboratory Animals”.

Conflict-of-interest statement: The authors declare no conflict of interest related to this study.

Data sharing statement: No additional data are available.

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Manuscript source: Unsolicited manuscript

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Received: September 20, 2016
Peer-review started: September 22, 2016
First decision: January 10, 2017
Revised: January 24, 2017
Accepted: February 16, 2017
Article in press: February 16, 2017
Published online: April 28, 2017

Abstract

AIM

To observe whether there are differences in the effects of electro-acupuncture (EA) and moxibustion (Mox) in rats with visceral hypersensitivity.

METHODS

EA at 1 mA and 3 mA and Mox at 43 ℃ and 46 ℃ were applied to the Shangjuxu (ST37, bilateral) acupoints in model rats with visceral hypersensitivity. Responses of wide dynamic range neurons in dorsal horns of the spinal cord were observed through the extracellular recordings. Mast cells (MC) activity in the colons of rats were assessed, and 5-hydroxytryptamine (5-HT), 5-hydroxytryptamine 3 receptor (5-HT3R) and 5-HT4R
expressions in the colons were measured.

RESULTS

Compared with normal control group, responses of wide dynamic range neurons in the dorsal horn of the spinal cord were increased in the EA at 1 mA and 3 mA groups (1 mA: 0.84 ± 0.74 vs 2.73 ± 0.65, P < 0.001; 3 mA: 1.91 ± 1.48 vs 6.44 ± 1.26, P < 0.001) and Mox at 43 ºC and 46 ºC groups (43 ºC: 1.76 ± 0.81 vs 4.14 ± 1.83, P = 0.001; 46 ºC: 5.19 ± 2.03 vs 7.91 ± 2.27, P = 0.01). MC degranulation rates and the expression of 5-HT, 5-HT3R and 5-HT4R in the colon of Mox 46 ºC group were decreased compared with model group (MC degranulation rates: 0.47 ± 0.56 vs 0.28 ± 0.78, P < 0.001; 5-HT: 1.42 ± 0.65 vs 7.38 ± 1.12, P < 0.001; 5-HT3R: 6.62 ± 0.77 vs 2.86 ± 0.88, P < 0.001; 5-HT4R: 4.62 ± 0.65 vs 2.22 ± 0.97, P < 0.001).

CONCLUSION

The analgesic effects of Mox at 46 ºC are greater than those of Mox at 43 ºC, EA 1 mA and EA 3 mA.

Key words: Electro-acupuncture; Moxibustion; Visceral hypersensitivity; Analgesic effect; Rats

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Core tip: Irritable bowel syndrome (IBS) is a common functional gastrointestinal disorder, and the visceral hypersensitivity is considered to be one of the most important factors in its pathogenesis. Both acupuncture and moxibustion can regulate visceral hypersensitivity in IBS; however, the underlying mechanisms remain unclear. The present study is designed to observe whether there are differences in the effects of electro-acupuncture with different current intensities and moxibustion at varying temperatures on visceral hypersensitivity and to explore the potential analgesic mechanisms of these two stimulations.

Zhao JM, Li L, Chen L, Shi Y, Li YW, Shang HX, Wu LY, Weng ZJ, Bao CH, Wu HG. Comparison of the analgesic effects of electro-acupuncture and moxibustion with visceral hypersensitivity rats in irritable bowel syndrome. World J Gastroenterol 2017; 23(16): 2928-2939. Available from: URL: http://www.wjgnet.com/1007-9327/full/v23/i16/2928.htm DOI: http://dx.doi.org/10.3748/wjg.v23.i16.2928

INTRODUCTION

Irritable bowel syndrome (IBS) is a common functional gastrointestinal disorder characterized by abdominal discomfort or pain associated with abnormal bowel movements. The pathogenesis of IBS is complex and has not been fully elucidated. However, current data suggest that it is associated with visceral hypersensitivity, altered gut motility and dysfunction of the brain-gut axis[1-4]. Recently, visceral hypersensitivity was considered to be one of the specific indicators for IBS, and it can, to some extent, induce symptoms, including urgent defecation, flatulence and abdominal pain[5]. Studies have shown that acupuncture can greatly regulate visceral hypersensitivity in IBS[6-8], but the underlying mechanism has not been identified. Other research has indicated that the stimulation from acupuncture and moxibustion can converge with visceral nociceptive afferent impulses in the spinal cord[9-11]. To provide experimental evidence for electro-acupuncture (EA) and moxibustion (Mox) in treating IBS with visceral hypersensitivity, this study attempted to observe the convergence of afferent impulse induced by EA with different current intensities and Mox at varying temperatures in wide dynamic range (WDR) neurons in the dorsal horns of the spinal cord, and the subsequent influence on activation of mast cells and changes in expression of 5-HT, 5-HT3R and 5-HT4R in the colon.

MATERIALS AND METHODS

Animals

Sprague-Dawley (SD) rats (male, Specific Pathogen Free, 250-300 g) were supplied by the Experimental Animal Center of the Chinese Academy of Traditional Chinese Medicine. Animals used in this study were treated according to Beijing Administration Rule of Laboratory Animal. All efforts were made to minimize the number of animals utilized and their suffering. All animal experiments in this study were performed under the guidelines approved by the Animal Ethics Committee of the China Academy of Traditional Chinese Medicine.

Visceral hypersensitivity model establishment

An experimental rat model of visceral hypersensitivity was established as previously described[12]. On the second day after the rats were fasted, the experiment was begun. Rats in the normal group were stimulated through manual manipulation around the anus, while rats in the other groups were stimulated by distending the colorectum (CRD). Daily CRD using an inflatable balloon (constructed from a latex glove finger, length 4 cm, inflated with air) attached to an intravenous line connected via a Y connector to a manual pump and a sphygmomanometer. The balloon was inserted into the colon while the animal was sedated, and CRD was induced while animals were fully awake. The distention was repeated twice daily at a 30-min interval. The rats were reared until they reached adulthood (at least 6 week old), and behavioral responses to visceral pain induced by acute CRD were then examined.

Groups and treatments

Fifty D-IBS rats were randomly assigned to five groups as follows: (1) EA 1 mA group (n = 10): needles
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Table 1  Withdrawal reflex scoring criteria

| Score | Description |
|-------|-------------|
| 0     | No behavioral response to colorectum |
| 1     | Immobile during distension of CR and occasional clicking the head at onset of the stimulus |
| 2 A   | A mild contraction of abdominal muscles, but no lifting of abdomen off the platform |
| 2 B   | A strong contraction of abdominal muscles and lifting of abdomen off the platform, but no lifting of pelvic structure off the platform |
| 3     | Arching body and lifting of pelvic structure and scrotum |

(0.22 mm diameter; 13 mm length, Hwato, Suzhou Medical Appliance Factory, Ltd, Suzhou, China) were inserted 3-5 mm into the skin at the ST37 (Shangjuxu, bilateral) acupoints, and each acupuncture needle was connected to a HANS-100 pain relieving apparatus (Nanjing Jisheng Medical Science and Technology, Ltd, Nanjing, China) with a stimulation frequency of 2.0 Hz and a stimulation intensity of 1.0 mA; (2) EA 3 mA group (n = 10): the treatment was the same as for the EA 1 mA group with a stimulation intensity of 3 mA; (3) Mox 43 °C group (n = 10): fine moxibustion made for 30 s of EA and Mox stimulation, the background activity was first recorded for 10 s. After 30 s of EA and Mox stimulation, the background activity was recorded again for 20 s.

Each acupuncture needle (0.25 mm × 25 mm) was connected to a HANS-100 pain relieving apparatus with a stimulation frequency of 2.0 Hz and a stimulation intensity of 1 mA and 3 mA. Mox stimulation at the temperatures of 43 °C ± 1 °C and 46 °C ± 1 °C were randomly applied. Both of EA and Mox were applied to Shangjuxu (ST37, bilateral) acupoints on the same side of the receptor field.

| Group | Description |
|-------|-------------|
| EA 1 mA | A stimulation frequency of 2.0 Hz and a stimulation intensity of 1.0 mA |
| EA 3 mA | A stimulation frequency of 2.0 Hz and a stimulation intensity of 1.0 mA |
| Mox 43 °C | A stimulation frequency of 2.0 Hz and a stimulation intensity of 1.0 mA |

Animal surgery

After anesthetizing with an intraperitoneal injection of urethane (10%, 1.0-1.2 g/kg), SD rats were fixed on the operating table in a prone position. The spinal cord at L1-L3 was exposed by removing the overlying musculature along the dorsal midline, and then spinal clamps were fixed on the vertebral plate. Spinal dura mater was carefully removed under a microscope to prevent damage to the medulla. After the surgery, a groove was sutured with the flap and then covered with liquid paraffin at 38 °C in order to keep the medulla wet. Following the insertion of micropipettes, 2% agar gel was placed over the surface of the medulla, not only to prevent the medulla from drying but also to stabilize the recordings by keeping the medulla fixed during respiration.

Extracellular recordings of WDR neuronal activity

The positions of the recorded sites were as follows: 0.5-1.5 mm lateral to the posterior median fissure of the spinal cord and 500-1500 μm beneath the surface of the medulla. During the recording, glass micropipettes (cusp: 5 μm, impedance: 8-12 MΩ) were inserted into nuclear groups to target WDR neurons. Through the micropipettes, single-unit activities were channeled into an 8301-micropipette amplifier (MEZ8301, Nihon Kohden) and Power Lab data collection system (PL4097, AD Instruments, Australia) for the amplification of cell discharge and signal management. The standard recording procedures were as follows: after neuronal discharges were stabilized, background activity was first recorded for 10 s. After 30 s of EA and Mox stimulation, the background activity was recorded again for 20 s.

Abdominal withdrawal reflex scores before and after treatment

The abdominal withdrawal reflex (AWR) scoring criteria are shown in Table 1, using a described procedure[12]. Rats in the treatment group were fasted beginning from the afternoon of the previous day. Vaseline was smeared on the surface of the balloon which was then slowly inserted into 5 cm of the rat anus according to the physical curve of the colorectum, and retained for 5 min. The test was initiated when the rats became adapted. After air was added into the balloon with a syringe, the rat rectum was stimulated and different degrees of contraction reactions were observed. The pressure (mmHg) during behavior responses, scored as 1, 2, 3, and 4, was recorded and expressed as the threshold of sensitivity (Table 1). Each score was tested three times, and each rat was tested by two persons who had not participated in the research design. Means were calculated (6 values in total). Three-minute intervals were used between each of the two tests to allow for full adaptation of rats.

Toluidine blue staining assay

Toluidine blue staining was used to stain MCs. First, de-paraffinization and hydration of 4-μm paraffin-embedded sections was achieved by soaking samples in xylene and ethanol. After 20 min each and then anhydrous graded ethanol was applied (90%, 80%, and 70% for 5 min each). Toluidine was then added to the tissue sections and stained for 20 min. Samples were washed in destilled water for 10 s and 0.5% acetic acid was added to differentiate color. Slides were...
Effects of Mox at different temperatures on the activation of WDR neurons in the dorsal horn of the spinal cord

Ten WDR neurons in the dorsal horn of the spinal cord for 11 control rats and 12 model rats, and the effects of EA with different current intensities on the WDR neurons were observed. It was found that the background activities of these neurons were activated by EA. Within the range of 1 mA and 3 mA, the degree of neuronal activation was increased concurrently with the increase in current intensity. When EA with 1 mA was applied, the degree of activation in the WDR neurons of normal rats was 0.84 ± 0.74 spikes/s, while that of model rats increased to 2.73 ± 0.65 spikes/s. There were statistically significant differences between the two groups of rats (P < 0.001). When the intensity increased to 3 mA, the degree of activation in the WDR neurons of normal rats was 1.91 ± 1.48 spikes/s, while that of model rats increased to 6.44 ± 1.26 spikes/s. There were also statistically significant differences between the two groups (P < 0.001). It is evident that EA with different intensities on ST37 acupoints has increased activation effects on WDR neurons in the dorsal horn of the spinal cord in model rats with visceral hypersensitivity, compared to normal rats (Figure 1).

Effects of Mox at different temperatures on the activation of WDR neurons in the dorsal horn of the spinal cord

Ten WDR neurons in the dorsal horn of the spinal cord for 11 normal rats and 13 model rats were recorded, respectively, and the effects of Mox at different temperatures on the WDR neurons were observed. The background activities of these neurons were activated by Mox. At a range of 43°C - 46°C, the degree of neuronal activation was increased concurrently with the increase in temperature. When Mox at 43°C was applied, the degree of activation in the WDR neurons of normal rats was 1.76 ± 0.81 spikes/s, while that of model rats increased to 4.14 ± 1.83 spikes/s. There were statistically differences between the two groups (P = 0.001). When the temperature increased to 46°C, the degree of activation in the WDR neurons of normal rats was 5.19 ± 2.03 spikes/s, while that of model rats increased to 7.91 ± 2.27 spikes/s. There were also statistically differences between the two groups (P = 0.01). It is evident that Mox applied at different temperatures to ST37 acupoints has increased activation effects on WDR neurons in the dorsal horn of the spinal cord in model rats with visceral hypersensitivity, compared with normal rats (Figure 2).
group at 20 mmHg, 40 mmHg and 80 mmHg were not significantly different from the NC group (20 mmHg: \( P = 0.265 \), 40 mmHg: \( P = 0.107 \), 80 mmHg: \( P = 0.063 \)), whereas AWR scores of the EA 3 mA group at 60 mmHg were different from the NC group (60 mmHg: \( P = 0.016 \)). These data indicated that Mox at 46 °C and EA at 1 mA treatments were able to decrease visceral hypersensitivity or increase the pain threshold significantly, and they were superior to the EA 3 mA group (Figure 3).

**Change of MC activity in colon**
Compared with the NC group, the MC counts in the colon were significantly increased in the Mox at 43 °C and 46 °C groups, and EA at 1 mA and 3 mA groups (\( P_{\text{Mox43}} < 0.001, P_{\text{Mox46}} < 0.001, P_{\text{Acu3mA}} = 0.007, P_{\text{Acu1mA}} < 0.001 \)). The MC degranulation rates in the colon were also significantly increased in the Mox at 43 °C and Mox at 46 °C and EA at 1 mA and 3 mA groups compared with the NC group (\( P < 0.001 \)). Compared with the MC group, the MC degranulation rates in the colon were significantly decreased in the Mox at 46 °C group (\( P < 0.001 \), and there were differences in MC degranulation rates between the Mox at 46 °C group and the Mox at 43 °C group (\( P = 0.005 \)) (Figures 4 and 5).

**5-HT expression in colon**
Compared with the MC group, 5-HT expressions in the colon were significantly decreased in the NC group, the Mox at 43 °C and 46 °C groups and the EA at 1 mA, 3 mA groups (\( P < 0.001 \), and the expressions in the Mox at 43 °C and 46 °C groups were significantly lower than in the EA at 1 mA, 3 mA groups (\( P_{\text{Mox43}} vs \text{Acu1mA} = 0.007, P_{\text{Mox43}} vs \text{Acu3mA} = 0.014, P_{\text{Mox46}} vs \text{Acu1mA} < 0.001, P_{\text{Mox46}} vs \text{Acu3mA} < 0.001 \)). 5-HT expression in the colon was not significantly different between the Mox 46 °C group and the NC group (\( P = 0.332 \)) (Figures 6 and 7).

**5-HT3R expression in colon**
Compared with the MC group, 5-HT3R expressions in the colon were significantly decreased in the NC group,
Figure 4  Change of mast cells activity in the colon. A: Number of mast cells in colonic mucosa, \(^{a}P < 0.05, ^{b}P < 0.01\), vs the MC group; \(^{c}P < 0.05, ^{d}P < 0.01\), vs the NC group; B: Mast cells degranulation(%) in colonic mucosa, \(^{b}P < 0.01\), vs the MC group; \(^{d}P < 0.01\), vs the NC group; \(^{e}P < 0.05\), \(^{f}P < 0.01\), vs the Mox 46 \(\degree C\) group.

Figure 5  Change of mast cells activity in the colon (black arrows indicate mast cells, red arrows indicate mast cell degranulation). A: Normal control group; B: Model control group; C: Mox at 43 \(\degree C\) group; D: Mox at 46 \(\degree C\) group; E: EA at 1 mA group; F: EA at 3 mA group.
the Mox at 43 °C and 46 °C groups and the EA at 3 mA group (P<0.001, P<0.001, P<0.001, P<0.001, P<0.001, P<0.001), and the expressions in the Mox at 43 °C and 46 °C groups were significantly reduced compared to the EA at 1 mA and 3 mA groups (P<0.001 vs Acu1mA < 0.001, P<0.001 vs Acu1mA < 0.001, P<0.001 vs Acu1mA < 0.001, P<0.001 vs Acu1mA < 0.001) (Figures 8 and 9).

**5-HT4R expression in colon**

Compared with the MC group, 5-HT4R expressions in the colon were significantly decreased in the NC group, the Mox at 43 °C and 46 °C groups and the EA at 1 mA and 3 mA groups (P<0.001, P<0.001, P<0.001, P<0.001, P<0.001, P<0.001). 5-HT4R expressions in the colon were not significantly different between the NC group and the Mox at 43 °C and 46 °C groups and the EA at 3 mA group (P<0.001, P<0.001, P<0.001, P<0.001, P<0.001, P<0.001), whereas there was a statistically significant difference between the NC group and EA 1 mA group (P<0.001). (Figures 10 and 11).

**DISCUSSION**

Studies have proven that acupuncture and moxibustion have analgesic effects[13,14]. Receptors in skin or muscles are activated by the mechanical stimulation of acupuncture and the heat stimulation of moxibustion, which affects nervous system and the biological activity of tissues and cells to relieve pains[15,16]. With the help of the supraspinal cord, afferent impulses induced by acupuncture and moxibustion can transmit along peripheral A or C fibers to the dorsal horn of the spinal cord, where they will converge and interact with the visceral nociceptive afferent impulse. Therefore, acupuncture and moxibustion can inhibit the response of neurons in the dorsal horn of the spinal cord activated by visceral nociceptive afferent impulses[11,17]. Afferent impulses of organs or deep tissues can sensitize related segmental neurons, which makes the impulse responses from the body surface more intense. Meanwhile, the size and function of acupoints can change with the visceral functions[18], and in pathological conditions, the function of an acupoint area in reflecting and treating disease can be greatly enhanced to reach the status of acupoints area sensitization[19]. Visceralgia and sensitization interact with each other. Therefore, the dorsal horn of the spinal cord is the key to regulation of visceral hypersensitivity. The function of acupoints is not only related to the sensitization in the condition of visceral hyperalgesia, but is also associated with the convergence of body surface afferent and visceral nociceptive afferents in the spinal cord or supraspinal cord[20]. Acupuncture and moxibustion transmit signals through the activation of receptors in acupoint areas or through bioactive substances, and the stimulation of acupuncture in the body surface can converge with visceral nociceptive stimuli in the spinal cord to regulate the body functions.

Our study found that while stimulating ST37 in model rats with visceral hypersensitivity, with the increase of EA intensities from 1 mA to 3 mA and Mox temperature from 43 °C to 46 °C, the reaction activation of WDR neurons in the dorsal horn of the spinal cord was increased. Meanwhile, the intestinal pain threshold of model rats with visceral hypersensitivity was markedly inhibited. The abnormal activation of mast cells that induce gut motility and secretion in the colon and abnormal expression of 5-HT, 5-HT3R and 5-HT4R were also significantly inhibited. This indicates that the analgesic effects of EA and/or Mox on the target tissues of model rats with visceral hypersensitivity not only have a positive correlation with their intensities, but are also associated with the fact that WDR neuronscurge and integrate the stimulation induced by EA and Mox. It has been reported that needing the acupoints near the pain spot or the adjacent vertebrae would achieve better analgesic effects[21]. Furthermore, the analgesic effects were achieved when Aδ fibers were activated, and they became stronger when type-C fibers were activated[22]. In this study, ST37 was selected as the location for EA and Mox application, and ST37 was located in the receptive field of WDR neurons that involved convergence and integration of visceral nociceptive afferent impulses[23]. We applied EA with 1 mA and 3 mA intensities, and Mox at 43 °C and 46 °C, to ST37 of normal rats and model rats respectively. It was found that EA with 3 mA and Mox at 46 °C can achieve more significant activation of WDR neurons in the dorsal horn of the spinal cord. Stimulation with low intensities, such as 1 mA EA and 43 °C Mox, can only activate type A neural fibers, whereas stimulation with high intensities, such as 3 mA EA and 46 °C Mox, can also activate type C fibers, thereby inducing greater analgesic effects.

Abnormal activation of mass cells in the colon is one of the main pathological characteristics of visceral hypersensitivity (including gut motility, abnormal secretion and visceral pain)[24,25]. In this study, the
MC counts and degranulation rates in the colons of all model rats (model group, 1 mA EA group, 3 mA EA group, 43 °C Mox group, 46 °C Mox group) were greatly increased compared with those of normal rats, which indicates that with visceral hypersensitivity, the counts of MCs in the colon are not only significantly increased, but also present abnormal activation (degranulation). Furthermore, degranulated MCs may release large quantities of active substances, such as histamine and 5-HT. According to several studies, as an important agent of regulating the brain-gut axis and the intestinal neural system, 5-HT can regulate visceral perception and visceral neural reflex. The activation of 5-HT3R can induce cellular pathways for non-specific positive ion movement, allowing the entry of Na⁺ and Ca²⁺ and the presence of K⁺. These pathways of positive ion movement can depolarize
postsynaptic neurons, transmit signals around and induce the release of excitatory or inhibitory neurotransmitters. In this way, smooth muscles may be contracted and relaxed abnormally\(^2\). In addition, 5-HT can influence gastrointestinal mobility through 5-HT4R, as activated 5-HT4R can play an active role in regulating gut mobility and visceral hyperalgesia through the induction of a release of acetylcholine or neurotransmitters for P motor nerves\(^2\),\(^3\).

In our study, we observed that after applying EA with different intensities and Mox at varying temperatures, degranulation rate of MC in the colons of rats with visceral hypersensitivity was reduced in varying degrees, which demonstrates that EA and Mox, especially the Mox at 46 ℃, have an inhibitory function towards the abnormally activated MCs in
areas with visceral hypersensitivity. At the same time, it was also found that the expression levels of 5-HT, 5-HT3R and 5-HT4R in model rats were much higher than those in normal group, as were the AWR scores. After stimulation of EA with 1 mA and 3 mA and Mox at 43 ℃ and 46 ℃ was performed, compared to the model group, the expression of 5-HT, 5-HT3R and 5-HT4R was decreased to varying degrees. Mox was more effective than EA in reducing the expression. Meanwhile, AWR scores for all treatment groups (especially the Mox at 46 ℃) were decreased markedly compared with the model group. These results indicate that in colons of rats with visceral hypersensitivity, MCs are activated abnormally and 5-HT, 5-HT3R and 5-HT4R have increased expression. Stimulating ST37 with EA with different intensities and Mox at varying temperatures can activate the WDR neurons in the dorsal horn of the spinal cord, thus relieving pains in the target organs (colons). Among four different stimulations, Mox at 46 ℃ has the best analgesic effects. Additionally, we also found that the analgesic effects were achieved by EA and Mox through WDR neurons of the spinal cord, which may affect the abnormal MC activation and high expression of 5-HT, 5-HT3R and 5-HT4R by converging and integrating the visceral nociceptive afferent impulse with stimulation induced by EA and Mox at the ST37 acupoints. These results may reveal one of the mechanisms of analgesic effects of acupuncture in treating IBS with visceral hypersensitivity.

There are differences between stimulation by EA with different current intensities and Mox at different temperatures in the relief of visceral hypersensitivity. The analgesic effects of Mox at 46 ℃ are much greater than those of Mox at 43 ℃, EA at 1 mA and EA at 3 mA. A potential mechanism is that WDR neurons from the dorsal horn of the spinal cord may converge and integrate the stimulation induced by EA and Mox in
the segmental receptor field with visceral nociceptive afferent impulses and then influence the MC activation reaction of the colon and the high expression of 5-HT, 5-HT3R and 5-HT4R.

ACKNOWLEDGMENTS

We are grateful to Bing Zhu, Pei-Jing Rong, Xin-Yan Gao from Institute of Acu-Mox, China Academy of Chinese Medical Sciences for their guidance on the electrophysiological experiments.

COMMENTS

Background

The pathogenesis of irritable bowel syndrome is complex and has not been fully elucidated. Recently, visceral hypersensitivity was considered to be one of the specific indicators for irritable bowel syndrome (IBS), and it can, to some extent, induce the symptoms, including urgent defecation, flatulence and abdominal pain. Acupuncture and moxibustion both can regulate visceral hypersensitivity in IBS, however, the underlying mechanisms remain unclear.

Research frontiers

Previous studies have shown that acupuncture can greatly regulate visceral hypersensitivity in IBS, this study attempted to provide experimental evidence for electro-acupuncture (EA) and moxibustion (Mox) in treating IBS with visceral hypersensitivity.

Innovations and breakthroughs

This study attempted to observe the convergence of afferent impulse induced by EA with different current intensities and Mox at varying intensities in wide dynamic range neurons in the dorsal horn of the spinal cord, and its influence on activation of mast cells and expression changes of 5-HT, 5-HT3R and 5-HT4R in colon.

Applications

To provide experimental evidence for EA and Mox in treating IBS with visceral hypersensitivity, and the results may reveal one of the mechanisms of analgesic effects of acupuncture in treating IBS with visceral hypersensitivity.

Peer-review

This manuscript is a well-conducted study with moderate relevance and novelty.

REFERENCES

1 Blomhoff S, Speitalen S, Jacobsen MB, Vatn M, Malt UF. Intestinal reactivity to words with emotional content and brain information processing in irritable bowel syndrome. Dig Dis Sci 2000; 45: 1160-1165 [PMID: 10877232]
2 Sagami Y, Shimada Y, Tayama J, Nomura T, Satake M, Endo Y, Shoji T, Karashisi K, Hongo M, Fukudo S. Effect of a corticotropic releasing hormone receptor antagonist on colonic sensory and motor function in patients with irritable bowel syndrome. Gut 2004; 53: 958-964 [PMID: 15194643]
3 Sinhamahapatra P, Saha SP, Chowdhury A, Chakrabarti SK, Ghosh A, Maiti B. Visceral afferent hypersensitivity in irritable bowel syndrome—evaluation by cerebral evoked potential after rectal stimulation. Am J Gastroenterol 2001; 96: 2150-2157 [PMID: 11467647 DOI: 10.1111.j.1572-0241.2001.03952.x]
4 Rüssel P, Pedersen P, Niddam D, Arendt-Nielsen L, Chen AC, Drewes AM. Cerebral response to electric stimulation of the colon and abdominal skin in healthy subjects and patients with irritable bowel syndrome. Scand J Gastroenterol 2001; 36: 1259-1266 [PMID: 11761014]
5 Zhou Q, Price DD, Caudle RM, Verne GN. Visceral and somatic hypersensitivity in a subset of rats following TNBS-induced colitis. Pain 2008; 134: 9-15 [PMID: 17481818 DOI: 10.1016/j.pain.2007.03.029]
6 Liu HR, Yang Y, Wu HG. Clinical study on acupuncture in treating diarrhea-predominant irritable bowel syndrome. Zhongjiu Xueye 2006; 8: 360-362 [DOI: 10.1007/s11726-008-0360-1]
7 Schneider A, Weiland C, Enck P, Joos S, Streitberger K, Maser-Gluth C, Zipfel S, Bagheri S, Herzog W, Friedrich HC. Neuroendocrinological effects of acupuncture treatment in patients with irritable bowel syndrome. Complement Ther Med 2007; 15: 255-263 [PMID: 18054727 DOI: 10.1016/j.ctim.2006.12.002]
8 Chu WC, Wu J, Yew DT, Zhang L, Shi L, Yeung DK, Wang D, Tong RK, Chan Y, Lao L, Leung PC, Berman BM, Sung JJ. Does acupuncture therapy after activation of neural pathway for pain perception in irritable bowel syndrome?: a comparative study of true and sham acupuncture using functional magnetic resonance imaging. J Neurogastroenterol Motil 2012; 18: 305-316 [PMID: 22837879 DOI: 10.3506/jnm.2012.18.3.305]
9 Yu LL, Li L, Rong PJ, Zhu B, Qin QG, Ben H, Huang GF. Changes in responses of neurons in spinal and medullary subnuclear reticularis dorsalis to acupoint stimulation in rats with visceral hyperalgesia. Evid Based Complement Alternat Med 2014; 2014: 768634 [PMID: 25552449 DOI: 10.1155/2014/768634]
10 Li L, Yu L, Rong P, Ben H, Li X, Zhu B, Chen R. Visceral nociceptive afferent facilitates function of subnuclear reticularis dorsalis to acupoint stimulation in rats. Evid Based Complement Alternat Med 2013; 2013: 931283 [PMID: 23762171 DOI: 10.1155/2013/931283]
11 Rong PJ, Zhu B, Huang QF, Gao XY, Ben H, Li YH. [Acupuncture inhibiting responses of spinal dorsal horn neurons induced by noxious dilation rectum and colon]. Zhongguo Zhen Jia 2005; 25: 645-650 [PMID: 16318154]
12 Al-Chaer ED, Kawasaki M, Pasricha PJ. A new model of chronic visceral hypersensitivity in adult rats induced by colon irritation during postnatal development. Gastroenterology 2000; 119: 1276-1285 [PMID: 11054385]
13 Palecek J, Palecova V, Willis WD. The roles of pathways in the spinal cord lateral and dorsal funiculi in signaling nociceptive somatic and visceral stimuli in rats. Pain 2002; 96: 297-307 [PMID: 11973002]
14 Liu HR, Qi L, Wu LY, Ma XP, Qin XD, Huang WY, Dong M, Wu HG. Effects of moxibustion on dynorphin and dynorphin in rats with chronic visceral hyperalgesia. World J Gastroenterol 2010; 16: 4079-4083 [PMID: 20731023 DOI: 10.3748/wjg.v16.i32.4079]
15 Zhang J, Zhang N. Study on mechanisms of acupuncture analgesia. Zhongguo Zhen Jia 2007; 27: 72-75
16 Huang R, Zhao J, Wu L, Dou C, Liu H, Weng Z, Lu Y, Shi Y, Wang X, Zhou C, Wu H. Mechanisms underlying the analgesic effect of moxibustion on visceral pain in irritable bowel syndrome: a review. Evid Based Complement Alternat Med 2014; 2014: 895914 [PMID: 25093032 DOI: 10.1155/2014/895914]
17 Bing Z, Villaneuva LE, Le Bars D. Acupuncture and diffuse noxious inhibitory controls: naloxone-reversible depression of activities of trigeminal convergent neurons. Neuroscience 1990; 37: 809-818 [PMID: 2247225]
18 Yu XC, Zhu B, Gao JH, Fu WX, Lu B, Cui HF, Qin LP. The scientific basis of the dynamic process of acupuncture. Zhongyi Zazhi 2007; 48: 971-973
19 Ziegler EA, Maguerl W, Meyer RA, Treede RD. Secondary hyperalgesia to punctate mechanical stimuli. Central sensitization to A-fibre nociceptor input. Brain 1999; 122 (Pt 12): 2245-2257 [PMID: 10581220]
20 Yu LL, Li L, Qin QG, Ben H, Rong PJ, Zhu B. Colorectal nociceptive signal input facilitates impact of acupoint stimulation of “Zusanli” (ST36) on electrical activities of wide dynamic range neurons in lumbar spinal cord in rats. Zhongyi Xianjia 2014; 39: 390-395
21 Zhu B, Rong PJ, Ben H, Gao XY, Li YQ. Study on the mechanism of acupuncture analgesia of segmental and systemic character. Zhenci Yanjiu 2007; 32: 1
Zhao JM et al. Comparison of EA and moxibustion

22 Rong PJ. Convergence and interaction between acupuncture signals and the inputs of visceral nociception. Beijing: Beijing University of Traditional Chinese Medicine, 2004

23 Zhu B. Systematic acupuncture and moxibustion. Beijing: People’s Medical Publishing House; 2015: 115

24 Barbara G, Stanghellini V, De Giorgio R, Cottrell GS, Santini D, Pasquinelli G, Morselli-Labate AM, Grady EF, Bunnell NW, Collins SM, Corinaldesi R. Activated mast cells in proximity to colonic nerves correlate with abdominal pain in irritable bowel syndrome. Gastroenterology 2004; 126: 693-702 [PMID: 14988823]

25 Shibata S. Novel cardiostimulant polypeptides (anthopleurin-A, B and C) isolated from sea anemone. Jpn J Pharmacol 1980; 30: 7P-9P [PMID: 7241857 DOI: 10.1053/j.gastro.2006.11.039]

26 Grundy D. 5-HT system in the gut: roles in the regulation of visceral sensitivity and motor functions. Eur Rev Med Pharmacol Sci 2008; 12 Suppl 1: 63-67 [PMID: 18924445]

27 Camilleri M. Serotonergic modulation of visceral sensation: lower gut. Gut 2002; 51 Suppl 1: i81-86 [PMID: 12077074]

28 Yan C, Xin-Guang L, Hua-Hong W, Jun-Xia L, Yi-Xuan L. Effect of the 5-HT4 receptor and serotonin transporter on visceral hypersensitivity in rats. Braz J Med Biol Res 2012; 45: 948-954 [PMID: 22832600]

29 Mader R, Kocher T, Haier J, Wieczorek G, Pfannkuche HJ, Ito M. Investigation of serotonin type 4 receptor expression in human and non-human primate gastrointestinal samples. Eur J Gastroenterol Hepatol 2006; 18: 945-950 [PMID: 16894306 DOI: 10.1097/01.meg.0000228975.87645.27]

P-Reviewer: Garg P  S-Editor: Ma YJ  L-Editor: Ma JY  E-Editor: Wang CH
