

Suspended moxibustion relieves chronic visceral hyperalgesia and decreases hypothalamic corticotropin-releasing hormone levels

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SM. Each treatment lasted for 30 min. Rats undergoing treatment with SM were not anesthetized. Untreated CVH rats and normal rats were used as controls. The abdominal withdrawal reflex was determined 30-90 min after the seven treatments. The hypothalamic corticotropin-releasing hormone (CRH) mRNA level was measured using real-time quantitative reverse transcription-polymerase chain reaction.

RESULTS: We found that SM treatment significantly decreased visceral sensitivity to colorectal distention in this rat model. In treated animals, SM also decreased the relative hypothalamic CRH mRNA expression level to control levels.

CONCLUSION: Lower hypothalamic CRH levels may mediate the beneficial effects of SM in this rat irritable bowel syndrome model.

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Key words: Chronic visceral hypersensitivity; Corticotropin-releasing hormone; Irritable bowel syndrome; Rat; Suspended moxibustion

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Abstract

AIM: To evaluate the effect of suspended moxibustion (SM) on rectal sensory thresholds and to analyze the possible mechanisms involved in SM treatment of chronic visceral hypersensitivity (CVH) in rats.

METHODS: SM was administered once daily to 37-d-old CVH rats for 7 d. The two pairs of acupoints (ST25 and ST37, bilateral) were simultaneously treated with

INTRODUCTION

Irritable bowel syndrome (IBS) is a functional disorder

characterized by chronic recurring abdominal pain or discomfort and altered bowel habits^[3]. Several recent studies have demonstrated that electro-acupuncture (EA) can decrease chronic visceral hypersensitivity (CVH) in a rat IBS model induced by mechanical colorectal irritation in the postnatal period^[2-6]. Previously, we reported that EA in this model can decrease hypothalamic corticotropin-releasing hormone (CRH) levels^[7]. CRH, a 41 amino acid peptide produced mainly in the paraventricular nucleus of the hypothalamus, is regarded as a major mediator of the stress response^[8]. IBS patients are reported to be hypersensitive to routine stress^[9], and stressful life events are known to contribute to the clinical course of IBS^[10].

Moxibustion is an alternative or complementary therapy that is also used to treat IBS. Methods of moxibustion include suspended moxibustion (SM, also known as warming moxibustion), scarring moxibustion and herb-partition moxibustion. Previously, we reported that both SM^[11] and herb-partition moxibustion^[12] could decrease CVH in a rat IBS model induced by mechanical colorectal irritation in the postnatal period. Moreover, we found that rats were relaxed or asleep during SM, indicating that the procedure was not stressful to the animals^[11]. Therefore, in the present study, we focused on whether SM could decrease hypothalamic CRH levels in rats.

MATERIALS AND METHODS

Animals

We used a rat model of CVH^[13] induced by mechanical colorectal irritation during postnatal development. Neonatal male Sprague-Dawley rats (5 d old) were obtained from the Experiment Animal Center, Shanghai University of Traditional Chinese Medicine. The animals were maintained in a plastic cage containing corn chip bedding at a controlled temperature (21°C) in a light-dark cycle (12 h:12 h). The maximum number of rats per cage was five. All rats were used strictly in accordance with the National Institutions of Health Guide for the Care and Use of Laboratory Animals.

Neonatal rats were subjected to daily mechanical colorectal distention (CRD) from the age of 8 d to the age of 21 d. Neonatal rats received CRD twice daily, at 30-min intervals using a procedure modified from previous studies^[6,13]. A balloon constructed from a condom (length 20.0 mm and diameter 3.0 mm) was inserted rectally into the descending colon. The balloon was distended with 0.5 mL air for 1 min. It was then deflated and withdrawn. The rats were reared until they reached adulthood (at least 6 wk old), and behavioral responses to visceral pain induced by acute CRD were then examined. SM was administered to CVH rats ($n = 8$) for 7 d. CVH rats without SM ($n = 8$) and normal rats ($n = 8$) were used as controls. After seven treatments, the abdominal withdrawal reflex (AWR) was monitored over a period of 30-90 min. The animals were then sacrificed by intraperitoneal anesthesia using sodium pentobarbital (80 mg/kg), and the hypothalamus was isolated immediately and frozen in liquid nitrogen.

SM treatment

In the CVH + SM group, one ignited moxa stick was suspended perpendicularly 2 cm above Tianshu (ST25)^[11] and Shangjuxu (ST37) (5 mm lateral to the anterior tubercle of the tibia and 20 mm below the knee joint). ST25 and ST37 were the two key acupoints chosen in this study based on our clinical treatment of patients with IBS since the 1980s. The two pairs of acupoints (ST25 and ST37) were simultaneously treated with SM. Each treatment consisted of 30 min of moxibustion (15 min for each pair of acupoints). SM was administered once daily to CVH rats for 7 d. The animals were not anesthetized before SM, but were held in the supine position in one gloved hand. Rats from both the normal group and CVH group were also held in one gloved hand in the supine position, but not treated with SM^[11], these were used as controls.

Colon stimulation and testing of the AWR

Behavioral responses to CRD in young adult rats were assessed by recording AWR scores, using a procedure modified from previous studies^[5,13]. After anesthesia with diethyl ether, a balloon (3 cm in length, made using one finger of a latex glove) was inserted into the descending colon. The rats were then housed in small lucite cubicles (20 cm × 8 cm × 8 cm) on a platform and allowed to wake up and adapt for 20 min. CRD was induced by rapidly inflating the balloon at pressures of 20, 40, 60 and 80 mmHg for a duration of 10 s. AWR scores were observed by two blinded observers using the scale developed by Al-Chaer *et al.*^[13].

Real-time quantitative reverse transcription-polymerase chain reaction

Total RNA was isolated from the hypothalamus using TRIZOL Reagent (TAKARA Biotechnology Co., Ltd., China), and quantified using a UV-3000 spectrophotometer (UNICO, USA). First-strand cDNA was synthesized using oligo(dT)15 primer, Moloney murine leukemia virus reverse transcriptase (TAKARA Biotechnology Co., Ltd., China), and 4 µL RNA. CRH and GAPDH (housekeeping gene) primers were designed in different exons to amplify cDNA using ABI Prism 7500 SDS Software (Applied Biosystems Co., Ltd., USA). CRH primers were: sense 5'-TGGCCTGCAGTGCAATGC-3' and antisense 5'-CCTGGCACTCAGAATAATTACAC-3'. Real-time quantitative polymerase chain reaction (qPCR) was performed with 5 µL of first-strand cDNA reaction in the presence of 0.5 µL dNTP, 10 µL specific buffer, 1 µL *Taq* polymerase, SYBR green fluorescent dye and the appropriate sense and antisense primers (0.5 µL) in a final volume of 50 µL (qPCR™ Core Kit, Shanghai DaWei'K Biology Technology Co., Ltd., China). PCR was carried out using the 7500 Sequence Detection System (Applied Biosystems). The reaction conditions were as follow: initial denaturation for 5 min at 95°C followed by 40 cycles with denaturation at 95°C for 15 s and annealing and extension at 60°C for 45 s. Three SYBR cycle threshold values were

Table 1 Abdominal withdrawal reflex scores in response to graded colorectal distention at 20, 40, 60 and 80 mmHg

Group	n	AWR score			
		20 mmHg	40 mmHg	60 mmHg	80 mmHg
Normal	8	0.13 ± 0.13	0.38 ± 0.18	0.75 ± 0.25	1.50 ± 0.33
CVH	8	1.38 ± 0.18 ^{b,d}	1.80 ± 0.25 ^{b,d}	2.75 ± 0.25 ^{b,d}	3.50 ± 0.27 ^{b,d}
CVH + SM	8	0.25 ± 0.16	0.50 ± 0.19	1.13 ± 0.30	1.63 ± 0.18

AWR: Abdominal withdrawal reflex; SM: Suspended moxibustion; CVH: Chronic visceral hypersensitivity. ^b*P* < 0.01 *vs* normal group; ^d*P* < 0.01 *vs* CVH + SM group.

averaged for each sample, and the RNA input for the target gene was calculated from the standard curve.

Statistical analysis

All values are expressed as mean ± SE. Statistical analyses were performed using one-way ANOVA followed by Fisher's PLSD procedure using SPSS 10.0 (SPSS Inc., USA). Dunnett's T3 test was used if variances were unequal. *P* < 0.05 was considered to be significant.

RESULTS

AWR scores in response to graded CRD at 20, 40, 60 and 80 mmHg

As shown in Table 1, the AWR scores in response to graded CRD (20, 40, 60, and 80 mmHg) in the normal control group were lower than in the CVH group (*P* < 0.01). SM treatment significantly reduced AWR scores in the CVH rats in response to CRD (20, 40, 60 and 80 mmHg).

Relative hypothalamic CRH mRNA expression

The relative CRH mRNA expression level was significantly higher in the CVH group than in the normal control group (*P* < 0.01). However, the relative CRH mRNA expression level was markedly lower in the CVH + SM group than in the normal control group (*P* < 0.01) (Table 2).

DISCUSSION

In our previous study, we found that SM depressed AWR scores following CRD stimulation at 20 mmHg. However, in this study SM depressed AWR scores following CRD stimulation at 20, 40, 60 and 80 mmHg, which may be due to the increased number of treatments (1 treatment *vs* 7 treatments), and acupoints (1 pair of acupoints *vs* 2 pairs of acupoints). Overall, the results of this experiment demonstrated the efficacy of SM in decreasing CVH in a rat IBS model induced by mechanical colorectal irritation in the postnatal period.

We previously reported that EA can decrease the hypothalamic CRH levels in a rat IBS model^[7]. Moreover, the rats were relaxed or asleep during SM, indicating that the procedure was not stressful to the animals^[11]. Stress is known to lead to central CRH release, and we have confirmed that hypothalamic CRH levels are elevated in IBS rats. In the present study, we focused on whether SM

Table 2 Relative hypothalamic corticotropin-releasing hormone mRNA expression

Group	n	CRH mRNA (relative expression)
Normal	8	0.29 ± 0.03
CVH	8	3.62 ± 0.23 ^{b,d}
CVH + SM	8	0.47 ± 0.06

CRH: Corticotropin-releasing hormone; SM: Suspended moxibustion; CVH: Chronic visceral hypersensitivity. ^b*P* < 0.01 *vs* normal group; ^d*P* < 0.01 *vs* CVH + SM group.

could decrease hypothalamic CRH expression level in rats. Our results showed that the relative hypothalamic CRH mRNA expression level also decreased in IBS rats, suggesting that the modulation of hypothalamic CRH may mediate the decreased visceral sensitivity arising from SM.

The effects of CRH on different tissues are mediated *via* CRH receptors on the cell membrane^[14]. CRH receptors are expressed in different brain regions^[15,16] and in several peripheral organs^[17]. Both central CRH receptor 1 (CRH-R1) and peripheral CRH-R1 are believed to be responsible for colorectal distension-induced sensitization^[18]. Moreover, the activation of CRH-R2 reduces visceral sensitivity induced by colorectal distension in conscious rats.

We hope that this study will pave the way for further studies on the relationship between CRH, CRH receptors, and SM. It will be necessary to determine whether SM can regulate the expression or activity of CRH receptors in IBS rats. Further experiments with CRH receptor antagonists would shed light on the functional relationship between SM and changes in CRH levels.

In conclusion, SM increased pain thresholds in a rat model of IBS and decreased relative hypothalamic CRH mRNA expression level. We suggest that reduced hypothalamic CRH levels may mediate the beneficial effects of SM in a rat IBS model induced by mechanical colorectal irritation in the postnatal period.

COMMENTS

Background

The authors previously reported that electro-acupuncture can decrease hypothalamic corticotropin-releasing hormone (CRH) levels in a rat model of irritable bowel syndrome (IBS). However, it is still not known whether suspended moxibustion (SM) can decrease hypothalamic CRH levels. Previously, the authors reported that both SM and herb-partition moxibustion can decrease chronic visceral hypersensitivity (CVH) in a rat IBS model induced by mechanical colorectal irritation in the postnatal period. Moreover, the authors found that rats were relaxed or asleep during SM, indicating that the procedure was not stressful to the animals.

Research frontiers

The effects of CRH on different tissues are mediated *via* CRH receptors on the cell membrane. CRH receptors are expressed in different brain regions and in several peripheral organs. Both central CRH receptor 1 (CRH-R1) and peripheral CRH-R1 are believed to be responsible for colorectal distension-induced sensitization. Moreover, the activation of CRH-R2 reduces visceral sensitivity induced by colorectal distension in conscious rats.

Innovations and breakthroughs

This study is the first to report on the effects of SM on hypothalamic CRH levels in CVH rats.

Terminology

Moxibustion is an alternative or complementary therapy and is also used to treat IBS. Methods of moxibustion include SM (also named warming moxibustion), scarring moxibustion and herb-partition moxibustion. In the SM treatment, one ignited moxa-stick was suspended perpendicularly above the acupoints.

Peer review

The paper is very interesting. But there are some questions to be addressed before publication.

REFERENCES

- 1 **Drossman DA**, Camilleri M, Mayer EA, Whitehead WE. AGA technical review on irritable bowel syndrome. *Gastroenterology* 2002; **123**: 2108-2131
- 2 **Wang XM**, Liu HR, Ding GH, Chen YF, Wu HA, Li N, Zhou EH, Qin XD, Yuan LS. Effects of electroacupuncture on c-Fos expression in the spinal cord and brain of rats with chronic visceral hypersensitivity. *Neural Regen Res* 2009; **4**: 339-343
- 3 **Liu HR**, Wang XM, Zhou EH, Shi Y, Li N, Yuan LS, Wu HG. Acupuncture at both ST25 and ST37 improves the pain threshold of chronic visceral hypersensitivity rats. *Neurochem Res* 2009; **34**: 1914-1918
- 4 **Chu D**, Cheng P, Xiong H, Zhang J, Liu S, Hou X. Electroacupuncture at ST-36 relieves visceral hypersensitivity and decreases 5-HT(3) receptor level in the colon in chronic visceral hypersensitivity rats. *Int J Colorectal Dis* 2010; Epub ahead of print
- 5 **Tian XY**, Bian ZX, Hu XG, Zhang XJ, Liu L, Zhang H. Electroacupuncture attenuates stress-induced defecation in rats with chronic visceral hypersensitivity via serotonergic pathway. *Brain Res* 2006; **1088**: 101-108
- 6 **Cui KM**, Li WM, Gao X, Chung K, Chung JM, Wu GC. Electroacupuncture relieves chronic visceral hyperalgesia in rats. *Neurosci Lett* 2005; **376**: 20-23
- 7 **Wu HG**, Liu HR, Zhang ZA, Zhou EH, Wang XM, Jiang B, Shi Z, Zhou CL, Qi L, Ma XP. Electroacupuncture relieves visceral sensitivity and decreases hypothalamic corticotropin-releasing hormone levels in a rat model of irritable bowel syndrome. *Neurosci Lett* 2009; **465**: 235-237
- 8 **Vale W**, Spiess J, Rivier C, Rivier J. Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and beta-endorphin. *Science* 1981; **213**: 1394-1397
- 9 **Whitehead WE**, Crowell MD, Robinson JC, Heller BR, Schuster MM. Effects of stressful life events on bowel symptoms: subjects with irritable bowel syndrome compared with subjects without bowel dysfunction. *Gut* 1992; **33**: 825-830
- 10 **Ferrier L**. Significance of increased human colonic permeability in response to corticotrophin-releasing hormone (CRH). *Gut* 2008; **57**: 7-9
- 11 **Zhou EH**, Liu HR, Wu HG, Shi Y, Wang XM, Tan LY, Yao LQ, Zhong YS, Jiang Y, Zhang LL. Suspended moxibustion relieves chronic visceral hyperalgesia via serotonin pathway in the colon. *Neurosci Lett* 2009; **451**: 144-147
- 12 **Zhou EH**, Liu HR, Wu HG, Shi Y, Wang XM, Yao LQ, Zhong YS, Yang Y. Herb-partition moxibustion relieves chronic visceral hyperalgesia and 5-HT concentration in colon mucosa of rats. *Neurol Res* 2009; **31**: 734-737
- 13 **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
- 14 **Chang CP**, Pearse RV 2nd, O'Connell S, Rosenfeld MG. Identification of a seven transmembrane helix receptor for corticotropin-releasing factor and sauvagine in mammalian brain. *Neuron* 1993; **11**: 1187-1195
- 15 **Primus RJ**, Yevich E, Baltazar C, Gallager DW. Autoradiographic localization of CRF1 and CRF2 binding sites in adult rat brain. *Neuropsychopharmacology* 1997; **17**: 308-316
- 16 **Lovenberg TW**, Liaw CW, Grigoriadis DE, Clevenger W, Chalmers DT, De Souza EB, Oltersdorf T. Cloning and characterization of a functionally distinct corticotropin-releasing factor receptor subtype from rat brain. *Proc Natl Acad Sci USA* 1995; **92**: 836-840
- 17 **Baigent SM**, Lowry PJ. mRNA expression profiles for corticotrophin-releasing factor (CRF), urocortin, CRF receptors and CRF-binding protein in peripheral rat tissues. *J Mol Endocrinol* 2000; **25**: 43-52
- 18 **Fukudo S**, Saito K, Sagami Y, Kanazawa M. Can modulating corticotropin releasing hormone receptors alter visceral sensitivity? *Gut* 2006; **55**: 146-148

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