World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i23.7181

World J Gastroenterol 2015 June 21; 21(23): 7181-7190
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Basic Study

Effects of electroacupuncture on corticotropin-releasing hormone in rats with chronic visceral hypersensitivity

Hui-Rong Liu, Xiao-Yi Fang, Huan-Gan Wu, Lu-Yi Wu, Jing Li, Zhi-Jun Weng, Xin-Xin Guo, Yu-Guang Li

Hui-Rong Liu, Xiao-Yi Fang, Yu-Guang Li, First Affiliated Hospital of Shantou University Medical College, Shantou 515041, Guangdong Province, China
Hui-Rong Liu, Huan-Gan Wu, Zhi-Jun Weng, Xin-Xin Guo, Shanghai Research Institute of Acupuncture and Meridian, Shanghai 200030, China
Lu-Yi Wu, Jing Li, Shanghai University of Traditional Chinese Medicine, Shanghai 201203, China

Author contributions: Liu HR, Fang XY and Wu HG contributed equally to this work; Liu HR, Fang XY and Wu LY analyzed the data and wrote the paper; Liu HR, Li J, Weng ZJ and Guo XX established the animal experimental model and participated equally in animal treatment; Liu HR, Weng ZJ and Wu LY performed the molecular investigations; Li YG and Wu HG designed and coordinated the research.

Supported by China Postdoctoral Science Foundation funded project, No.200801260, No.20080430117; the National Basic Research Program of China 973 program, No.2009CB522900; and the Specialized Research Fund for the Doctoral Program of Higher Education No. 20123107110008.

Ethics approval: The study was reviewed and approved by the Shanghai University of Traditional Chinese Medicine Institutional Review Board.
Institutional animal care and use committee: All procedures involving animals were reviewed and approved by the Institutional Animal Care and Use Committee of Shanghai University of Traditional Chinese Medicine.
Conflict-of-interest: The authors declare that there is no conflict of interest regarding the publication of this paper.
Data sharing: Technical appendix, statistical code, and dataset available from the corresponding author at lhr_tcm@139.com. Participants gave informed consent for data sharing.

Correspondence to: Yu-Guang Li, PhD, Professor, First Affiliated Hospital of Shantou University Medical College, 57 Changping Road, Shantou 515041, Guangdong Province, China. yglee317@126.com
Telephone: +86-754-88854536

Received: January 6, 2015
Peer-review started: January 6, 2015
First decision: February 10, 2015
Revised: February 26, 2015
Accepted: April 17, 2015
Article in press: April 17, 2015
Published online: June 21, 2015

Abstract

AIM: To investigate the effect of electroacupuncture on corticotropin-releasing hormone (CRH) in the colon, spinal cord, and hypothalamus of rats with chronic visceral hypersensitivity.

METHODS: A rat model of chronic visceral hypersensitivity was generated according to the internationally accepted method of colorectal balloon dilatation. In the 7th week after the procedure, rats were randomly divided into a model group (MG), electroacupuncture group (EA), and sham electroacupuncture group (S-EA). After treatment, the abdominal withdrawal reflex (AWR) score was used to assess the behavioral response of visceral hyperalgesia. Immunohistochemistry (EnVision method), ELISA, and fluorescence quantitative PCR methods were applied to detect the expression of CRH protein and mRNA in the colon, spinal cord, and hypothalamus.

RESULTS: The sensitivity of the rats to the colorectal distension stimulus applied at different strengths

(20-80 mmHg) increased with increasing stimulus strength, resulting in increasing AWR scores in each group. Compared with NG, the AWR score of MG was significantly increased ($P < 0.01$). After conducting EA, the AWR scores of the rats were decreased compared with MG rats. The relative expression of CRH mRNA in the colon, spinal cord, and hypothalamus of MG rats was significantly increased compared with NG rats ($P < 0.01$). CRH mRNA in the colon and spinal cord of EA and S-EA rats was decreased to varying degrees ($P > 0.05$) compared with normal rats (NG). However, the decrease in EA compared with MG rats was statistically significant ($P < 0.01$). The average optical density of CRH expression in the colon of the MG rats was significantly enhanced compared with NG ($P < 0.05$), while the average optical density of CRH expression in the EA and S-EA rats was significantly decreased compared with MG rats ($P < 0.01$, $P < 0.05$, respectively). Compared with MG rats, the CRH concentration in the spinal cord of EA rats was significantly reduced ($P < 0.01$), but there was no significant change in S-EA rats ($P > 0.05$).

CONCLUSION: Electroacupuncture at the Shangjuxu acupoint was able to significantly reduce the visceral hypersensitivity in rats, and regulated the expression of CRH protein and mRNA in the colon, spinal cord and hypothalamus at different levels, playing a therapeutic role in this model of irritable bowel syndrome.

Key words: Corticotropin-releasing hormone; Electroacupuncture; Irritable bowel syndrome; Visceral pain; Shangjuxu

Core tip: Visceral hypersensitivity is enhanced in irritable bowel syndrome (IBS) rats. Electroacupuncture at the Shangjuxu acupoint can significantly reduce the visceral hypersensitivity to colorectal distension in IBS rats, with a reduced pain threshold being observed. The expression of corticotropin-releasing hormone (CRH) protein and mRNA in the target organ and central nervous system of these rats is abnormal to varying degrees, and electroacupuncture at can regulate the expression of CRH protein and mRNA in the target organ (colon) and central nervous system (spinal cord and hypothalamus), exerting a therapeutic effect on visceral hypersensitivity in IBS rats.

Liu HR, Fang XY, Wu HG, Wu LY, Li J, Weng ZJ, Guo XX, Li YG. Effects of electroacupuncture on corticotropin-releasing hormone in rats with chronic visceral hypersensitivity. *World J Gastroenterol* 2015; 21(23): 7181-7190 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i23/7181.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i23.7181

INTRODUCTION

Irritable bowel syndrome (IBS) is a common gastrointestinal disease characterized by chronic and recurrent lower abdominal pain and discomfort as well as altered bowel habits. However, there are no associated organic changes. Because of its high incidence, IBS causes major economic losses to both patients and society. The drug therapies applied in modern medicine primarily focus on symptomatic treatment, and no effective drug is currently available to treat this condition. In Traditional Chinese Medicine, acupuncture therapy has a long history, and there is a rich clinical experience of using this method to treat syndromes similar to IBS. Acupuncture can effectively inhibit chronic visceral hypersensitivity during growth and development in rats, and is a potentially effective treatment for IBS[1-3]. Our research group has many years of clinical experience in the treatment of IBS using acupuncture therapy[4-6], and the Shangjuxu acupoint (ST 37) is one of the key acupoints commonly used in clinical practice. The Shangjuxu acupoint is the lower confluent point of the large intestine meridian and is a major acupoint targeted in the clinical treatment of abdominal pain and diarrhea.

Visceral hypersensitivity is considered an important pathophysiological characteristic of IBS, whose pathological mechanism is associated with a variety of neurotransmitters[7,8]. Acupuncture plays a regulatory role in relation to a number of related neurotransmitters[9-11], among which hypothalamic corticotropin-releasing hormone (CRH) is a major transmitter of the stress response in the brain-gut axis, playing an important role in the occurrence of visceral hypersensitivity in IBS[12-14]. With a deepening understanding of the mechanism of action of acupuncture treatment for IBS, it has been demonstrated that electroacupuncture can regulate the expression of hypothalamic CRH in IBS[15,16]. This study was performed to investigate the regulatory effect of electroacupuncture at the Shangjuxu acupoint on visceral hypersensitivity in IBS rats and its impact on the expression of CRH and its receptors in the target organ (colon) and central nervous system (spinal cord and hypothalamus). From this perspective, the functional mechanism of electroacupuncture at the Shangjuxu acupoint in the treatment of visceral hypersensitivity in IBS was elucidated, followed by an in-depth analysis of the connotations of treatment of IBS *via* the Shangjuxu acupoint in neurobiology.

MATERIALS AND METHODS

Animal care and use

The animal protocol was designed to minimize pain or discomfort to the animals. A total of six litters of male, newborn Sprague-Dawley rats (5 d after birth)

of specific-pathogen-free grade were selected. There were 5 to 8 neonatal rats in each litter. Every litter of rat pups was co-reared with a lactating female rat, and the lactating rats were fed food and water *ad libitum*. The rats were reared under a 12 h light/dark cycle, at room temperature of 20 ± 2 ℃ and a relative humidity of 50%-70%. Neonatal rats that showed no adverse reactions after three days were included in the experiment. All experiments on animals were performed according to the guidelines established by the International Association for the Study of Pain (IASP).

Eight-day-old neonatal rats were randomly divided into a normal group (NG) and model group (MG). NG rats were raised normally, without any stimulation, while the neonatal rats in MG were subjected to colorectal distension (CRD) stimulation. The preparation of the rat model with chronic visceral hypersensitivity was performed according to the procedure of Al-Chaer *et al*[17]. As detailed in this procedure, a small amount of liquid paraffin was first smeared on the balloon surface, which was then slowly inserted from the anus along the physiological curvature of the rat rectum at a depth of approximately 2 cm, to reach the descending colon of the rat. The balloon was subsequently inflated to a volume of approximately 0.2 mL with a syringe and left inflated for 1 min before the air was released. The same stimulus was applied again after 1 h. After the stimulation was complete, the balloon was removed. This modeling procedure was performed once a day for 14 d. All animals were euthanized by barbiturate overdose (intravenous injection, 150 mg/kg pentobarbital sodium) for tissue collection.

Behavioral response

The abdominal withdrawal reflex (AWR) score was semi-quantitatively evaluated in rats which received CRD stimulation after 6 wk of continuous rearing following the modeling procedure. Two NG rats and two MG rats were also randomly selected for histological colon observations to determine whether the model was successful. Within 90 min after treatment was complete, CRD stimulation and AWR scoring were performed on the rats. The balloon was slowly inserted through the anus into the colon, and CRD stimuli of 4 different strengths (20, 40, 60 and 80 mmHg) were applied, with each CRD stimulation lasting for 20 s, with an interval of 5 min before the next stimulation. Stimulation at each strength was repeated 3 times. The AWR scores were obtained by 2 different technicians. The average (integer) of the AWR score based on the observation of each CRD stimulation served as the AWR score for the CRD stimulation at that strength.

The standard for AWR scoring was based on the AWR scoring system of Al-Chaer *et al*[17]: 0: no behavioral response to CRD; 1: the rat occasionally turned its head at the beginning of the stimulus and remained motionless during CRD; 2: slight contraction was observed in the abdominal muscles, with no abdominal lift; 3: strong contraction was observed in the abdominal muscles, with abdominal lift, but no pelvic or scrotum lift; and 4: the body was arched, with pelvis and scrotum lift.

Groups and treatment

Based on a confirmed successful model, in the 7th week after the modeling procedure, the MG rats were randomly divided into MG, electroacupuncture (EA) group and sham-EA (S-EA) group to conduct the therapeutic intervention, while the NG rats served as the normal control group. The NG and MG rats did not receive treatment, and the same fixation procedure was performed as in the treatment groups. For EA, electroacupuncture was performed at the bilateral Shangjuxu acupoint of the rats, with a 0.25 mm needle with a length of 13 mm being introduced to a depth of 5 mm. The acupuncture procedure was manipulated with a connected Han's Acupoint Nerve Stimulator to provide a sparse-dense wave at a frequency of 2/100 Hz for 20 min once daily for 7 d. For S-EA, electroacupuncture was performed at the bilateral Shangjuxu acupoint of the rats, with a 0.25 mm needle with a length of 13 mm introduced to a depth of 5 mm. The acupuncture stimulus was not performed in this case, but this procedure also lasted for 20 min, once daily for 7 d.

Preparation for ELISA before the experiment

After adding 100 μL of a standard and 100 μL of a sample that had been appropriately diluted to the corresponding well of the plate (R and D systems, United States), the plate was incubated for 20 min and then washed, and 100 μL of biotin anti-rat CRH was then added to each well. After washing, 100 μL of TMB coloration solution was added. The OD value at 450 nm was read within 15 min (Thermo Multiskan-MK3, Finland). CRH (ng/mL) = the concentration determined from the standard curve × the dilution factor of the sample.

Fluorescence quantitative PCR

TRIzol (Life Technologies, Carlsbad, CA, United States) was used to acquire total RNA from the tissues according to the manufacturer's protocol. Total RNA was extracted from tissue homogenates. The sample concentrations, purities and integrities were also determined. A total RNA equivalent was used to reverse-transcribe cDNA *via* real-time PCR using the SYBRGreen PCR kit (Shanghai Ruijie Biological Engineering Company, Shanghai, China). ABI Prism 7500 SDS software was used to analyze the mRNA expression level of the target gene. The relative mRNA expression of a target gene =, $2^{-\Delta\Delta CT}$ × 100%; ΔCT = the CT value of the target gene - the CT value of the internal reference (GAPDH). The sequences of primers were as follows: β-actin 5'- CCGAGGGCCCACTAAAGG

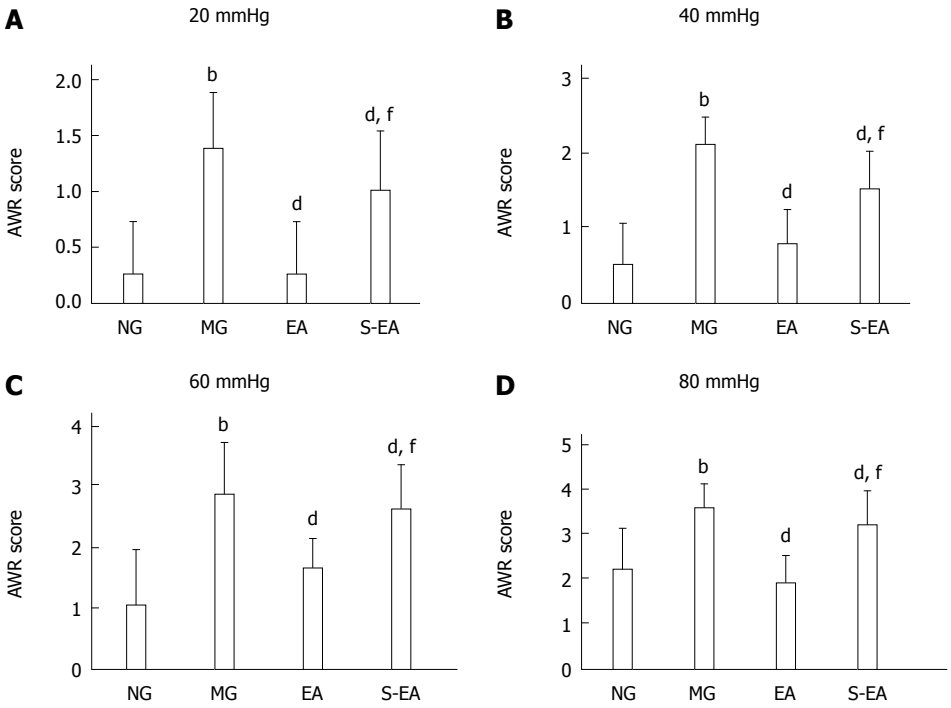

Figure 1 Comparison of the abdominal withdrawal reflex scores of the rats in each group. Under colorectal distension stimulation at the same strength; $^{b}P < 0.01$ *vs* NG; $^{d}P < 0.01$ *vs* MG; $^{f}P < 0.01$ *vs* EA. NG: Normal group; MG: Model group; EA: Electroacupuncture group; S-EA: Sham EA group; AWR: Abdominal withdrawal reflex.

-3′(sense), 5'- GCTGTTGAGTCACAGGAGCAA-3′ (antisense); CRH 5'- TGGCCTGCAGTGCAATGC -3′(sense), 5'- CCTGGCACTCAGAATAATTCACAC-3′(antisense).

Detection of CRH expression in the colon via immunohistochemistry

Tissue sections were deparaffinized and rehydrated in water, then heated for antigen retrieval. After endogenous peroxidase was inhibited with 0.3% H_2O_2, a primary antibody against CRH (Abcam Ltd., Hong Kong, China) was added dropwise to an appropriate dilution, followed by incubation at 37 ℃ for 2 h. The working solution of the DAB agent (Gene Tech Co., Ltd., Shanghai, China) for EnVision color development was added dropwise, and the developing time was controlled under a light microscope. The slides were dehydrated, cleaned, and mounted for observation.

Statistical analysis

The statistical analysis of all experimental data was performed using SPSS 18.0 statistical software (IBM Corp., Armonk, NY, United States). The quantitative data showing a normal distribution in the normality test are presented as the mean ± standard deviation (SD). The differences between groups were compared using one-way analysis of variance (ANOVA). The least significant difference method was applied for comparisons between groups in the case of homogeneity of variance, while Dunnett's T3 was applied for comparisons between groups in the case of heterogeneity of variance. $P < 0.05$ was considered statistically significant. The data not normally distributed are presented as M (Q_{25}-Q_{75}), and ANOVA was performed after rank transformation. The statistical methods of this study were reviewed by Xiao-Yi Fang from Shantou University Medical College.

RESULTS

AWR scores of the rats in each group after treatment

To evaluate changes in visceral sensitivity caused by CRD in rats , as well as the therapeutic effect of electroacupuncture, we measured AWRs to CRD after electroacupuncture treatment. In this study, internationally accepted AWR scoring was used to determine the change in the sensitivity to visceral pain. As shown in Figure 1, the sensitivities of the rats to CRD stimulation at strengths of 20 mmHg, 40 mmHg, 60 mmHg, and 80 mmHg were different. With an increasing stimulus strength, the AWR scores of each group also increased. As shown in Figure 1A-D, the AWR score of MG was increased significantly compared with NG rats ($P < 0.01$). For the rats in S-EA and EA groups, compared with MG, the AWR scores of the rats were decreased, but the difference was significant only for EA rats ($P < 0.05$). The AWR score was significantly lower in EA compared with S-EA rats ($P < 0.01$). The results suggested that electroacupuncture at the Shangjuxu acupoint could effectively increase the pain threshold of rats with chronic visceral hypersensitivity.

Positive expression of CRH in the colons of the rats

In the above experiment, electroacupuncture effectively relieved IBS visceral pain in rats with visceral hypersensitivity. To determine whether this

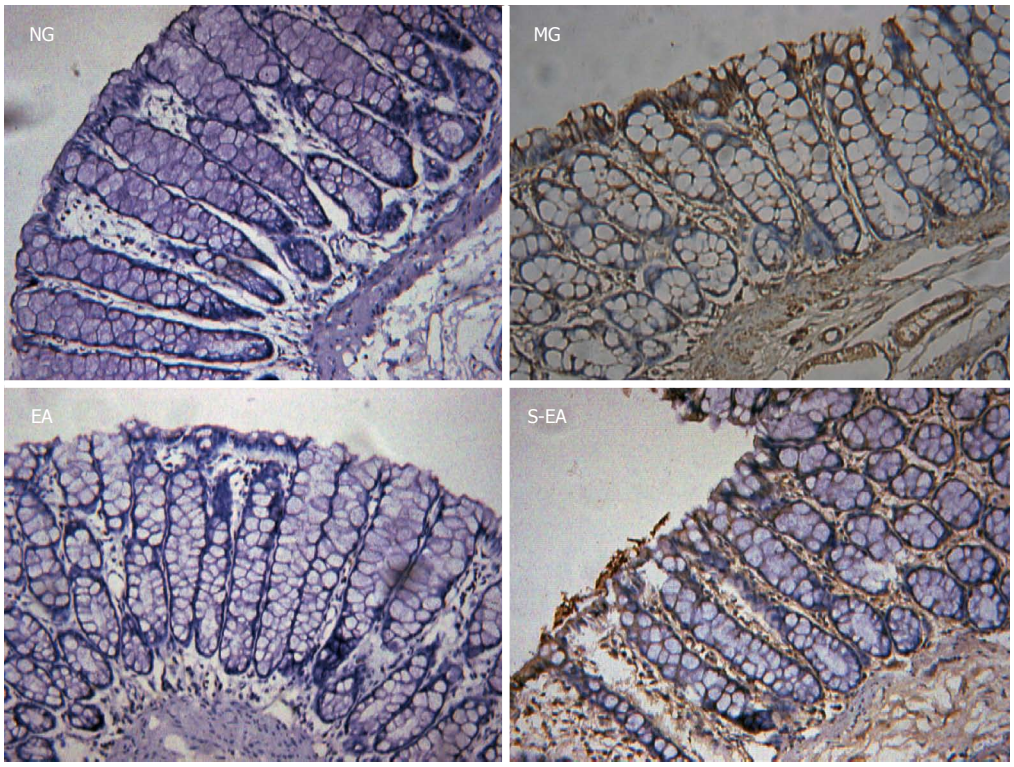

Figure 2 Expression of corticotropin-releasing hormone in the colon tissue of the rats in each group (magnification × 200). NG: Normal group; MG: Model group; EA: Electroacupuncture group; S-EA: Sham EA group.

Table 1 Average optical density of the positive target expression of corticotropin-releasing hormone in the colon tissue of the rats in each group

Group	*n*	Average optical density of the positive target expression of CRH
NG	8	0.3792 (0.3328-0.4272)
MG	8	0.5044 (0.4283-0.5498)[a]
EA	8	0.3743 (0.3524-0.4032)[d]
S-EA	8	0.3818 (0.3349-0.4256)[c]

[a]$P < 0.05$ *vs* NG; [c]$P < 0.05$ *vs* MG; [d]$P < 0.01$ *vs* MG. CRH: Corticotropin-releasing hormone; NG: Normal group; MG: Model group; EA: Electroacupuncture group; S-EA: Sham EA group.

Table 2 Average optical density of the positive target expression of corticotropin-releasing hormone in the spinal cord and hypothalamus of the rats in each group

Group	*n*	Positive expression of CRH in the spinal cord (ng/L)	Positive expression of CRH in the hypothalamus (ng/L)
NG	8	104.8787 (97.3855-107.4352)	50.3548 (44.8451-62.7847)
MG	8	115.4573 (113.2975-116.6474)[a]	99.9861 (97.3855-102.3663)[a]
EA	8	107.0385 (106.5536-108.0523)[b]	63.8866 (54.0573-66.9280)[b]
S-EA	8	110.1239 (107.0826-112.9889)[a]	84.9556 (68.7351-100.6032)[a,c]

[a]$P < 0.05$ *vs* NG; [b]$P < 0.01$ *vs* MG; [c]$P < 0.05$ *vs* EA. NG: Normal group; MG: Model group; EA: Electroacupuncture group; S-EA: Sham EA group.

effect was achieved by regulating the expression of CRH protein in the colon, we performed further investigations by means of immunohistochemistry. CRH was expressed in the mucosa epithelium, stroma, epithelial proximal glands, submucosa, and muscularis, as shown in Figure 2. CRD apparently upregulated CRH expression in NG rats, similar to previous studies. Compared with NG, the average optical density of CRH expression in the colon of the rats in MG was significantly enhanced ($P < 0.05$). Compared with MG, the average optical density of CRH expression in EA and S-EA was significantly reduced ($P < 0.01$, $P < 0.05$), suggesting that electroacupuncture at the Shangjuxu acupoint could reduce the average optical density of CRH expression in the colon of the IBS rats, as shown in Table 1.

CRH content in the spinal cord and hypothalamus of rats in each group

Although the above experiment indicated that CRH protein levels in the peripheral colon were involved in modulation of visceral pain by electroacupuncture, whether CRH levels in the central nervous system also played a role remain unknown. Therefore, we measured the expression of CRH protein in the spinal cord and hypothalamus. Compared with NG rats, the content of CRH in the spinal cord of MG rats was significantly enhanced ($P < 0.01$). Compared with MG rats, the content of CRH in the spinal cord in EA rats was significantly reduced ($P < 0.01$), while S-EA rats showed no significant change ($P > 0.05$), suggesting that electroacupuncture at the Shangjuxu acupoint could reduce the abnormally elevated expression of CRH in the spinal cord of IBS rats, as shown in

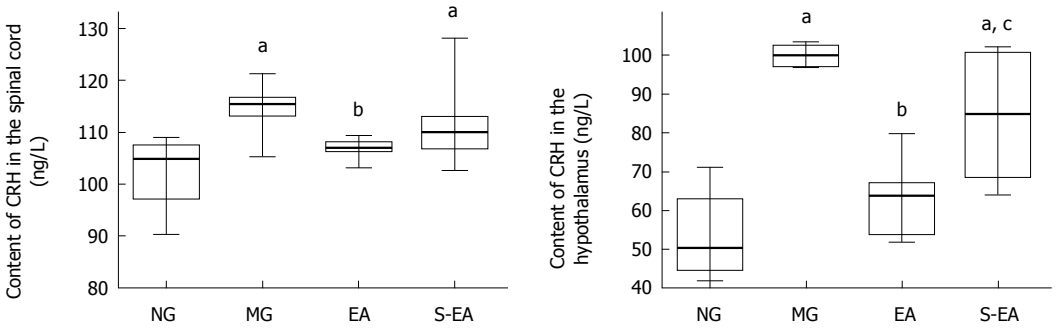

Figure 3 Corticotropin-releasing hormone content in the spinal cord and hypothalamus of the rats in each group. $^{a}P < 0.05$ *vs* NG; $^{b}P < 0.01$ *vs* MG; $^{c}P < 0.05$ *vs* EA. NG: Normal group; MG: Model group; EA: Electroacupuncture group; S-EA: Sham EA group.

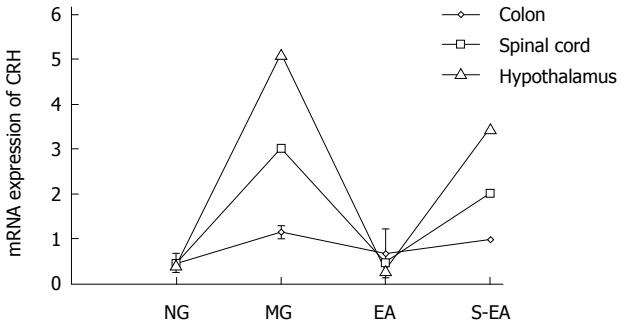

Figure 4 mRNA expression of corticotropin-releasing hormone in the colon, spinal cord, and hypothalamus of the rats in each group. NG: Normal group; MG: Model group; EA: Electroacupuncture group; S-EA: Sham EA group.

Figure 3A and Table 2. Similar findings were obtained in the hypothalamus. Compared with NG rats, the CRH content in the hypothalamus of MG rats was significantly enhanced ($P < 0.01$). Compared with MG rats, the CRH content in the hypothalamus of EA rats was significantly reduced ($P < 0.01$), while S-EA rats showed no significant change ($P > 0.05$), suggesting that electroacupuncture at the Shangjuxu acupoint could downregulate the abnormally elevated expression of CRH in the hypothalamus of IBS rats, as shown in Figure 3B and Table 2. There are changes in the expression of CRH from the peripheral to the central colon, which may be an important pathway of electroacupuncture analgesia, but more evidence is needed to prove this specificity.

mRNA expression of CRH in the colon, spinal cord, and hypothalamus: Fluorescence quantitative PCR

In the peripheral colon and central spinal cord and hypothalamus, CRH is involved in the modulation of visceral pain in rats with IBS. Meanwhile, electroacupuncture has a significant analgesic effect which depends on CRH protein expression. Whether CRH mRNA also changes has not been determined, hence, the expression of CRH mRNA in colon, spinal cord and hypothalamus was measured (Figure 4). Compared with NG rats, the relative mRNA expression of CRH in the colon of the MG rats was significantly enhanced ($P < 0.01$), while the expression in EA and S-EA rats decreased to varying degrees ($P > 0.05$ compared with NG rats). Compared with MG rats, the expression in EA rats was significantly reduced ($P < 0.01$), while no statistically significant difference could be detected between S-EA and MG rats. These results suggest that electroacupuncture at the Shangjuxu acupoint could downregulate the abnormally elevated expression of CRH mRNA in the colon of IBS rats. The relative expression of CRH mRNA in the spinal cord of the rats in each group showed an overall non-normal distribution. Compared with NG rats, the relative expression of CRH mRNA in the spinal cord of MG rats was significantly enhanced ($P < 0.01$), whereas it was significantly reduced in EA rats ($P < 0.01$ compared with MG rats), and there was no significant difference compared with NG rats ($P > 0.05$). These results suggest that electroacupuncture at the Shangjuxu acupoint was able to downregulate the abnormally elevated expression of CRH mRNA in the spinal cord of IBS rats. The relative expression of CRH mRNA in the hypothalamus of the rats in each group showed an overall non-normal distribution. Compared with NG rats, the relative expression of CRH mRNA in the hypothalamus of MG rats was significantly enhanced ($P < 0.01$), whereas it was significantly reduced in EA rats ($P < 0.01$ compared with MG rats). Additionally, a reduction was observed in S-EA rats compared with MG ($P < 0.05$), though the difference was still significant compared with NG rats ($P < 0.01$). These results suggest that electroacupuncture at the Shangjuxu acupoint could significantly downregulate the abnormally elevated expression of CRH mRNA in the hypothalamus of IBS rats. The detailed data are shown in Table 3.

DISCUSSION

IBS is a common digestive disease with clinical manifestations of abdominal pain and bloating, as well as altered bowel habits and stool abnormalities. In recent years, abnormal visceral hypersensitivity has come to be considered an important pathophysiological characteristic of IBS. The manifestations of a decreased pain threshold and increased sensitivity of the rectum in IBS patients have been confirmed by large amounts of clinical and experimental data. However, the

Table 3 mRNA expression of corticotropin-releasing hormone in the colon, spinal cord, and hypothalamus of the rats in each group

Group	*n*	Colon	Spinal cord	Hypothalamus
NG	8	0.4656 ± 0.2948	0.4684 (0.3211-0.6661)	0.4174 (0.3540-0.5941)
MG	8	1.1474 ± 0.2220[a]	3.0358 (1.8806-3.5932)[a]	5.1351 (4.4917-5.2711)[a]
EA	8	0.6667 ± 0.1415[d]	0.4748 (0.2972-1.0029)[a]	0.2892 (0.2181-0.3513)[a]
S-EA	8	0.9861 ± 0.5395	2.0223 (1.4990-2.9099)[a]	3.4474 (3.3225-3.6267)[a,d,e]

[a]$P < 0.05$ *vs* NG; [c]$P < 0.05$, [d]$P < 0.01$ *vs* MG; [e]$P < 0.05$ *vs* EA. NG: Normal group; MG: Model group; EA: Electroacupuncture group; S-EA: Sham EA group.

neurobiological mechanism of visceral hypersensitivity in IBS has not yet been fully elucidated. In recent years, the pathophysiological characteristics of visceral hypersensitivity in patients with IBS have been attracting increasing attention. A clinical study revealed that the capacity to cause bowel sensitivity in patients with IBS and their pain threshold are significantly lower than those of normal people, indicating visceral hypersensitivity[18]. IBS patients show a significantly reduced tolerance of balloon dilatation in the colon and rectum, displaying increased pain intensity[19]. Some scholars believe that this sensitivity may be related to an enhanced sensation in the peripheral or central nervous system, and it may also be related to abnormalities in the processing and integration of the incoming signal in the brain[20]. The interactions between the brain and gut are achieved through the brain-gut axis. A variety of stressors affecting the central nervous system and gut stimulate the brain-gut axis, which will subsequently regulate the response caused by the stressors through the individual or combined effects of multiple mechanisms involving neural pathways, immunology and endocrinology[21-23], ultimately resulting in visceral sensory abnormalities[24]. In recent years, studies have found that the Shangjuxu acupoint can regulate the motility, absorption and secretion of the large and small intestines, exhibiting a 2-way modulatory effect on gastrointestinal function. This acupoint can also be targeted to treat diarrhea or constipation and other intestinal bowel dysfunction syndromes with a satisfactory efficacy[25-27]. Our research team has confirmed the efficacy of acupoints such as Shangjuxu in the treatment of IBS in previous clinical studies, and it can also regulate the immune function of patients[28,29]. Therefore, studying the application of the theory of electroacupuncture at the Shangjuxu acupoint (lower confluent point of large intestine meridian) in the treatment of IBS and its neurobiological connotations is of significance.

Abnormalities in the visceral sensory pathway may lead to visceral hypersensitivity, including excessive sensitization at the level of spinal cord, an abnormal response in the cortex, and a deficiency in the pain suppression system. In addition, stress/anxiety may be involved in the development of visceral hypersensitivity, and hypothalamic CRH is a key factor in this interaction, though the limbic system and the locus coeruleus (LC)-norepinephrine system also play an extremely important role. The distribution of CRH-R1 is consistent with the distribution of CRH, while CRH-R2 is located in the heart, skeletal muscle, gastrointestinal tract, and epithelial cells in cerebral blood vessels and the choroid plexus[30]. The distribution of CRH and its receptors in the gastrointestinal tract suggests that they may be closely associated with IBS. Experimental results showed that the levels of corticosteroids and the inflammatory cytokines IL-6 (in conjunction with its soluble receptor) and IL-8 are elevated in all patients with IBS (exhibiting diarrhea and constipation, alternating between the two conditions). Following CRH stimulation, an increase in the release of corticosteroids in IBS patients can be observed[31], suggesting that elevated release of CRH may increase the incidence of IBS. While under stress, the specific movements of the intestinal efferent autonomic nervous system of both IBS patients and healthy control subjects were found to be altered, but only IBS patients showed an increased visceral sensation[32]. A recent clinical study demonstrated that intravenous injection of a CRH receptor antagonist, α-helical CRH, could significantly reduce diarrhea-predominant visceral hypersensitivity in IBS patients[33]. Additionally, outer peripheral injection of CRH can improve sensitivity to balloon dilatation in the colon and reduce the pain threshold of patients, similar to the visceral hypersensitivity observed in IBS[34], and the release of CRH in the central nervous system caused by anxiety and stress may also enhance intestinal sensitivity to pain[35]. Animal studies have shown that visceral hypersensitivity induces significant expression of CRH in the hypothalamus of IBS rats[36,37]. CRH was weakly expressed in the brain and spinal cord of control rats and highly expressed in the rat model of visceral hypersensitivity. Furthermore, in sections of brain, positive CRH expression was widely detected in the hypothalamus, and the lower part of the third ventricle also showed a high level of expression. In sections of the spinal cord, positive neurons also exhibited significant expression, but the distribution of CRH-positive neurons was sparser than in sections of brain, and the area of positive expression was decreased compared with brain sections. When under stress, CRH regulates behavior and autonomic responses through binding to different parts of the CRH-R1 and CRH-R2 receptors. Some studies have shown that the CRH-R1 signaling pathway is associated with stress-related changes in the function of the colon and visceral hypersensitivity[38]. CRH stimulates colonic motility and causes visceral hyperalgesia, mainly through interacting with CRH-R1[33,39]. Recently, it was reported that the application of non-selective

CRH receptor antagonists may improve the changes in colonic motility and the pain response evoked by rectal electrical stimulation in IBS patients[40].

A large number of clinical and fundamental studies have demonstrated that acupuncture plays a specific conditioning and balancing role in the physiological functions and metabolic activity of each system and organ in the body *via* the corresponding acupoint. This conditioning and balancing role is the basis for the treatment of all diseases using acupuncture. Acupuncture can produce a variety of benign bidirectional regulatory effects on the body. This regulation is achieved through a series of biological changes produced after the acupuncture signal activates the neuroendocrine system, which is reflected in various systems, such as the respiratory, digestive and cardiovascular systems[15,16]. As the center of endocrine regulation and autonomic nerves, the hypothalamus represents the ultimate common pathway for the activities occurring in the hypothalamic-pituitary-adrenal (HPA) axis. Zhu *et al*[41] experimentally investigated the impact of electroacupuncture at the Shangjuxu acupoint on CRH/CRH-R1 mRNA levels in the LC neurons, showing that restraint stress could increase the contents of CRH in LC neurons and simultaneously upregulate the expression of CRH-R1 mRNA in LC neurons, leading to an increase in the synthesis of CRH-R1, while electroacupuncture at the Shangjuxu acupoint could both reduce the release of CRH in LC neurons and downregulate the expression of CRH-R1 mRNA to interfere with the synthesis of CRH-R1, thereby inhibiting the binding of CRH to CRH1R and weakening the CRH-induced colon stress response. In this study, an internationally recognized animal model of IBS chronic visceral hypersensitivity was employed[17], and the results showed that the AWR score of MG rats was abnormally elevated, indicating the existence of visceral hypersensitivity in the IBS model rats, with a reduced pain threshold being observed. Electroacupuncture at the Shangjuxu acupoint can effectively reduce the abnormally elevated pain threshold of the rats with chronic visceral hypersensitivity, and play a therapeutic role.

The results of this study showed that the average optical density of CRH expression in the colon and the CRH concentration in the spinal cord and hypothalamus of the rats with IBS were abnormally elevated, and the average optical densities of CRH expression in EA and S-EA were significantly reduced. The CRH concentrations in the spinal cord and hypothalamus of the rats in EA were significantly reduced, with no significant change being observed in S-EA. These results suggest that electroacupuncture at the Shangjuxu acupoint can lower the abnormally elevated expression and content of CRH in the target organ (colon) and central nervous system (spinal cord and hypothalamus) of IBS rats. Compared with NG rats, the relative expression levels of CRH mRNA in the colon, spinal cord, and hypothalamus of MG rats were significantly increased, whereas these levels were significantly decreased after electroacupuncture treatment, showing significant differences compared with MG rats. In contrast, expression in S-EA rats did not change significantly. These results suggest that electroacupuncture at the Shangjuxu acupoint can downregulate the abnormally elevated expression of CRH mRNA in the colon, spinal cord, and hypothalamus of IBS rats.

COMMENTS

Background

Irritable bowel syndrome (IBS) is a common gastrointestinal disease characterized by chronic and recurrent lower abdominal pain and discomfort as well as altered bowel habits. The drug therapies applied in modern medicine primarily focus on symptomatic treatment, and no effective drug is currently available to treat this condition. Acupuncture therapy has a long history, and there is rich clinical experience in using this method to treat syndromes similar to IBS. This study was performed to investigate the regulatory effect of electroacupuncture at the Shangjuxu acupoint on visceral hypersensitivity in IBS rats and its impact on the expression of corticotropin-releasing hormone (CRH) and its receptors in the target organ and central nervous system. From this perspective, the functional mechanism of electroacupuncture at the Shangjuxu acupoint in the treatment of visceral hypersensitivity in IBS was elucidated.

Research frontiers

Visceral hypersensitivity is considered an important pathophysiological characteristic of IBS, whose pathological mechanism is associated with a variety of neurotransmitters. Acupuncture plays a regulatory role in relation to a number of related neurotransmitters, among which hypothalamic CRH is a major transmitter of the stress response in the brain-gut axis, playing an important role in the occurrence of visceral hypersensitivity in IBS. With the deepening understanding of the mechanism of action of acupuncture treatment for IBS, it has been demonstrated that electroacupuncture can regulate the expression of hypothalamic CRH in IBS.

Innovations and breakthroughs

CRH is a major transmitter of the stress response in the brain-gut axis, playing an important role in the occurrence of visceral hypersensitivity in IBS. From this perspective, the functional mechanism of electroacupuncture at the Shangjuxu acupoint in the treatment of visceral hypersensitivity in IBS was elucidated, followed by an in-depth analysis of the connotations of treatment of IBS *via* the Shangjuxu acupoint in neurobiology.

Applications

The functional mechanism of electroacupuncture at the Shangjuxu acupoint in the treatment of visceral hypersensitivity in IBS was elucidated. The electroacupuncture can downregulate the abnormally elevated expression of CRH mRNA in the colon, spinal cord, and hypothalamus of IBS rats, and provides a scientific basis for clinical acupuncture treatment in IBS.

Peer-review

In this paper, the authors aimed to investigate the levels of corticotropin-releasing hormone in the colon and CNS at different levels when regulated by electroacupuncture for the treatment of rats with irritable bowel syndrome. CRH is a classical major mediator of the stress response in the brain-gut axis.

REFERENCES

1 **Ma XP**, Hong J, An CP, Zhang D, Huang Y, Wu HG, Zhang CH, Meeuwsen S. Acupuncture-moxibustion in treating irritable bowel syndrome: how does it work? *World J Gastroenterol* 2014; **20**: 6044-6054 [PMID: 24876727 DOI: 10.3748/wjg.v20.i20.6044]

2 **Okada K**, Kawakita K. Analgesic action of acupuncture and moxibustion: a review of unique approaches in Japan. *Evid Based Complement Alternat Med* 2009; **6**: 11-17 [PMID: 18955231 DOI: 10.1093/ecam/nem090]

3 **Shi XM**. [Indications and prospects of acupuncture-moxibustion].

Zhongguo Zhenjiu 2011; **31**: 961-964 [PMID: 22136013]
4 **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 [PMID: 19108755 DOI: 10.1179/174313209X382313]
5 **Wu LY**, Bao CH, Ge LB, Zhou CL, Liu HR, Qi L, Yi T, Wu HG, Wang XM. Mild moxibustion at Tianshu (ST 25) decreases expression of prokineticin-1 and prokineticin receptor-1 in colon tissue of rats with chronic visceral hyperalgesia. *Neural Regeneration Research* 2011; **6**: 2600-2604 [DOI: 10.3969/j.issn.1673-5374.2011.33.007]
6 **Zhou EH**, Wang XM, Ding GH, Wu HG, Qi L, Liu HR, Zhang SJ. Suspended moxibustion relieves chronic visceral hyperalgesia and decreases hypothalamic corticotropin-releasing hormone levels. *World J Gastroenterol* 2011; **17**: 662-665 [PMID: 21350717 DOI: 10.3748/wjg.v17.i5.662]
7 **Kanazawa M**, Hongo M, Fukudo S. Visceral hypersensitivity in irritable bowel syndrome. *J Gastroenterol Hepatol* 2011; **26** Suppl 3: 119-121 [PMID: 21443723 DOI: 10.1111/j.1440-1746.2011.06640.x]
8 **Qiao LY**. Neurotrophin signaling and visceral hypersensitivity. *Front Biol* (Beijing) 2014; **9**: 216-224 [PMID: 25484893]
9 **Yi T**, Qi L, Wu HG, Ma XP, Liu HR, Wang XM, "Analgesic action of suspended moxibustion in rats with chronic visceral hyperalgesia correlates with enkephalins in the spinal cord". *Neural Regeneration Research* 2012; **3**: 219-222 [DOI: 10.3969/j.issn.1673-5374.2012.03.011]
10 **Liu HR**, Qi L, Wu LY, Ma XP, Qin XD, Huang WY, Dong M, Wu HG. Effects of moxibustion on dynorphin and endomorphin in rats with chronic visceral hyperalgesia. *World J Gastroenterol* 2010; **16**: 4079-4083 [PMID: 20731023 DOI: 10.3748/wjg.v16.i32.4079]
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 [PMID: 19114087 DOI: 10.1016/j.neulet.2008.12.026]
12 **Muscatello MR**, Bruno A, Scimeca G, Pandolfo G, Zoccali RA. Role of negative affects in pathophysiology and clinical expression of irritable bowel syndrome. *World J Gastroenterol* 2014; **20**: 7570-7586 [PMID: 24976697 DOI: 10.3748/wjg.v20.i24.7570]
13 **Tayama J**, Sagami Y, Shimada Y, Hongo M, Fukudo S. Effect of alpha-helical CRH on quantitative electroencephalogram in patients with irritable bowel syndrome. *Neurogastroenterol Motil* 2007; **19**: 471-483 [PMID: 17564629 DOI: 10.1111/j.1365-2982.2007.00903.x]
14 **Ma XP**, Tan LY, Yang Y, Wu HG, Jiang B, Liu HR, Yang L. Effect of electro-acupuncture on substance P, its receptor and corticotropin-releasing hormone in rats with irritable bowel syndrome. *World J Gastroenterol* 2009; **15**: 5211-5217 [PMID: 19891022 DOI: 10.3748/wjg.15.5211]
15 **Fukudo S**. Role of corticotropin-releasing hormone in irritable bowel syndrome and intestinal inflammation. *J Gastroenterol* 2007; **42** Suppl 17: 48-51 [PMID: 17238026 DOI: 10.1007/s00535-006-1942-7]
16 **Wu HG**, Liu HR, Zhang ZA, Zhou EH, Wang XM, Jiang B, Shi Z, Zhou CL, Qi L, Ma XP. Electro-acupuncture relieves visceral sensitivity and decreases hypothalamic corticotropin-releasing hormone levels in a rat model of irritable bowel syndrome. *Neurosci Lett* 2009; **465**: 235-237 [PMID: 19765402 DOI: 10.1016/j.neulet.2009.09.018]
17 **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 DOI: 10.1053/gast.2000.19576]
18 **Dong WS**, Zou DW, Li ZS, Xu GM, Zou XP, Zhu AY, Yi N, Man XH. Mechanism of Visceral hypersensitivity in patients with irritable bowel syndrome. *Zhonghua Xiaohua Zazhi* 2004; **24**: 8
19 **Simrén M**, Abrahamsson H, Björnsson ES. An exaggerated sensory component of the gastrocolonic response in patients with irritable bowel syndrome. *Gut* 2001; **48**: 20-27 [PMID: 11115818 DOI: 10.1136/gut.48.1.20]
20 **Lan L**. The relation of visceral hypersensitivity induced by anxiety and serum corticosterone level in rats and the effect and signification of anti-anxiety treatment on both. *Zhengzhou Daxue Shushi Lunwen* 2005; **1**: 8-10
21 **Hyland NP**, O'Mahony SM, O'Malley D, O'Mahony CM, Dinan TG, Cryan JF. Early-life stress selectively affects gastrointestinal but not behavioral responses in a genetic model of brain-gut axis dysfunction. *Neurogastroenterol Motil* 2015; **27**: 105-113 [PMID: 25443141 DOI: 10.1111/nmo.12486]
22 **Karling P**, Norrback KF, Adolfsson R, Danielsson A. Gastrointestinal symptoms are associated with hypothalamic-pituitary-adrenal axis suppression in healthy individuals. *Scand J Gastroenterol* 2007; **42**: 1294-1301 [PMID: 17852841 DOI: 10.1080/00365520701395945]
23 **Lee KN**, Lee OY. Intestinal microbiota in pathophysiology and management of irritable bowel syndrome. *World J Gastroenterol* 2014; **20**: 8886-8897 [PMID: 25083061 DOI: 10.3748/wjg.v20.i27.8886]
24 **Holzer P**, Farzi A. Neuropeptides and the microbiota-gut-brain axis. *Adv Exp Med Biol* 2014; **817**: 195-219 [PMID: 24997035 DOI: 10.1007/978-1-4939-0897-4_9]
25 **Xiong F**, Wang Y, Li SQ, Tian M, Zheng CH, Huang GY. Clinical study of electro-acupuncture treatment with different intensities for functional constipation patients. *J Huazhong Univ Sci Technolog Med Sci* 2014; **34**: 775-781 [PMID: 25318892 DOI: 10.1007/s11596-014-1351-8]
26 **Xu X**, Zheng C, Zhang M, Wang W, Huang G. A randomized controlled trial of acupuncture to treat functional constipation: design and protocol. *BMC Complement Altern Med* 2014; **14**: 423 [PMID: 25351765 DOI: 10.1186/1472-6882-14-423]
27 **Liu Z**, Liu J, Zhao Y, Cai Y, He L, Xu H, Zhou X, Yan S, Lao L, Liu B. The efficacy and safety study of electro-acupuncture for severe chronic functional constipation: study protocol for a multicenter, randomized, controlled trial. *Trials* 2013; **14**: 176 [PMID: 23768191 DOI: 10.1186/1745-6215-14-176]
28 **Qi L**, Liu HR, Yi T, Wu LY, Liu XR, Zhao C, Shi Y, Ma XP, Wu HG. Warming Moxibustion Relieves Chronic Visceral Hyperalgesia in Rats: Relations to Spinal Dynorphin and Orphanin-FQ System. *Evid Based Complement Alternat Med* 2013; **2013**: 920675 [PMID: 23573158 DOI: 10.1155/2013/920675]
29 **Liu HR**, Qi L, Wang XL, Guan YH, Zuo CT, Tan LY, Yuan LS, Ma XP, Wang XM, Zhou EH, Wu HG. Electroacupuncture at Tianshu (ST 25) for diarrhea-predominant irritable bowel syndrome using positron emission tomography Changes in visceral sensation center. *Neural Regen Res* 2010; **5**: 1220-1225 [DOI: 10.3969/j.issn.1673-5374.2010.16.004]
30 **Zhang MJ**, Zhang QY. Corticotropin-releasing factor and corticotropin-releasing factor receptor. *Guojibingli Kexue Yu Linchang Zazhi* 2006; **2**: 162-165
31 **Dinan TG**, Quigley EM, Ahmed SM, Scully P, O'Brien S, O'Mahony L, O'Mahony S, Shanahan F, Keeling PW. Hypothalamic-pituitary-gut axis dysregulation in irritable bowel syndrome: plasma cytokines as a potential biomarker? *Gastroenterology* 2006; **130**: 304-311 [PMID: 16472586 DOI: 10.1053/j.gastro.2005.11.033]
32 **Murray CD**, Flynn J, Ratcliffe L, Jacyna MR, Kamm MA, Emmanuel AV. Effect of acute physical and psychological stress on gut autonomic innervation in irritable bowel syndrome. *Gastroenterology* 2004; **127**: 1695-1703 [PMID: 15578507 DOI: 10.1053/j.gastro.2004.08.057]
33 **Taché Y**, Martinez V, Wang L, Million M. CRF1 receptor signaling pathways are involved in stress-related alterations of colonic function and viscerosensitivity: implications for irritable bowel syndrome. *Br J Pharmacol* 2004; **141**: 1321-1330 [PMID: 15100165 DOI: 10.1038/sj.bjp.0705760]
34 **Lembo T**, Plourde V, Shui Z, Fullerton S, Mertz H, Tache Y, Sytnik B, Munakata J, Mayer E. Effects of the corticotropin-releasing factor (CRF) on rectal afferent nerves in humans. *Neurogastroenterol Motil* 1996; **8**: 9-18 [PMID: 8697187 DOI: 10.1111/j.1365-2982.1996.tb00237.x]

35 **Howard Mertz**. Altered CNS processing of visceral pain in IBS. In: Michael Camilleri, Robin C Spiller. Irritable Bowel Syndrome. *Diag Treat* 2002; 55-68
36 **Chao G**, Lv B, Meng L, Zhang S, Zahng L, Guo Y. [Influence of tongxie prescription on CRF expression in spinal cord and brain of hypersensitive viscera rats]. *Zhongguo Zhongyao Zazhi* 2010; **35**: 2012-2016 [PMID: 20931858 DOI: 10.4268/cjcmm20101522]
37 **Chao GQ**, Lv B, Meng LN, Zhang S,Zhang L,Guo Y.Effect of Changyanning Tangjiang on the expression of corticotropin releasing factor in spinal cord and brain of rats with visceral hypersensitivity. *Shijie Huaren Xiaohua Zazhi* 2008; **16**: 2112-2118
38 **Sato N**, Suzuki N, Sasaki A, Aizawa E, Obayashi T, Kanazawa M, Mizuno T, Kano M, Aoki M, Fukudo S. Corticotropin-releasing hormone receptor 1 gene variants in irritable bowel syndrome. *PLoS One* 2012; **7**: e42450 [PMID: 22957021 DOI: 10.1371/journal.pone.0042450]
39 **Chao GQ**, Lv B. Relationship between brain CRF and IBS. *Guoji Xiaohuabing Zazhi* 2008; **28**: 371-373
40 **Sagami Y**, Shimada Y, Tayama J, Nomura T, Satake M, Endo Y, Shoji T, Karahashi K, Hongo M, Fukudo S. Effect of a corticotropin releasing hormone receptor antagonist on colonic sensory and motor function in patients with irritable bowel syndrome. *Gut* 2004; **53**: 958-964 [PMID: 15194643 DOI: 10.1136/gut.2003.018911]
41 **Zhu QY**. Central mechanisms of electroacupuncture ST 37 on rat with colonal disfunetion induced acute restraint stress. Hu Bei University of Traditional Chinese Medicine PhD thesis, 2008

P- Reviewer: Kadiyska TK **S- Editor**: Qi Y
L- Editor: Cant MR **E- Editor**: Liu XM

Published by **Baishideng Publishing Group Inc**
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com

ISSN 1007-9327