NEURAL REGENERATION RESEARCH
Volume 8, Issue 9, March 2013

www.nrronline.org

doi:10.3969/j.issn.1673-5374.2013.09.004 [http://www.nrronline.org; http://www.sjzsyj.org]
Weng ZJ, Wu LY, Lu Y, Wang LD, Tan LY, Dong M, Xin YH. Electroacupuncture diminishes $P2X_2$ and $P2X_3$ purinergic receptor expression in dorsal root ganglia of rats with visceral hypersensitivity. Neural Regen Res. 2013;8(9):802-808.

Electroacupuncture diminishes $P2X_2$ and $P2X_3$ purinergic receptor expression in dorsal root ganglia of rats with visceral hypersensitivity★

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Abstract

Electroacupuncture at *Shangjuxu* (ST37) and *Tianshu* (ST25) can improve visceral hypersensitivity in rats. Colorectal distension was used to establish a rat model of chronic visceral hypersensitivity. Immunohistochemistry was used to detect $P2X_2$ and $P2X_3$ receptor expression in dorsal root ganglia from rats with chronic visceral hypersensitivity. Results demonstrated that abdominal withdrawal reflex scores obviously increased following establishment of the model, indicating visceral hypersensitivity. Simultaneously, $P2X_2$ and $P2X_3$ receptor expression increased in dorsal root ganglia. After bilateral electroacupuncture at *Shangjuxu* and *Tianshu*, abdominal withdrawal reflex scores and $P2X_2$ and $P2X_3$ receptor expression decreased in rats with visceral hypersensitivity. These results indicated that electroacupuncture treatment improved visceral hypersensitivity in rats with irritable bowel syndrome by reducing $P2X_2$ and $P2X_3$ receptor expression in dorsal root ganglia.

Key Words

neural regeneration; acupuncture and moxibustion; $P2X_2$; $P2X_3$; visceral hypersensitivity; irritable bowel syndrome; electroacupuncture; P2 purinergic receptors; abdominal withdrawal reflex scores; acupuncture and moxibustion; peripheral nerve injury; grants-supported paper; photographs-containing paper; neuroregeneration

Research Highlights

(1) $P2X_2$ and $P2X_3$ receptors participated in visceral hypersensitivity in primary afferent neurons of dorsal root ganglia in rats.
(2) Electroacupuncture reduced visceral hypersensitivity in rats with chronic visceral hypersensitivity.
(3) Electroacupuncture improved visceral hypersensitivity in rats with chronic visceral hypersensitivity by regulating $P2X_2$ and $P2X_3$ receptor expression in dorsal root ganglia.

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Received: 2012-06-18
Accepted: 2013-02-22
(N20120419003/YJ)

INTRODUCTION

Chronic visceral hypersensitivity is a pathophysiological characteristic of irritable bowel syndrome[1-2]. Each level in the brain-gut axis, *i.e*. neurotransmitters in the periphery, spinal cord, and central nervous system, all participate in the occurrence of visceral hypersensitivity[3-5]. Sensory signals in the intestinal tract are transferred into the central nervous system mainly through nociceptive and somatic pathways. Nociceptive sensations are transferred into the posterior horn of the spinal cord *via* sympathetic fibers, and then into somatic sensation projection areas *via* an afferent pathway in the spinal cord. Physiological

sensations from the gastrointestinal tract are transferred to the central nervous system *via* the vagus nerve allowing adjustment of gastrointestinal secretory and motor functions[6]. Visceral sensations are then transferred to secondary sensory neurons through nodose ganglion and spinal dorsal root ganglion. Of these, common visceral sensations are transferred *via* nodose ganglion, but afferent pain sensations are propagated *via* dorsal root ganglion[7-8].

Adenosine triphosphate transfers information *via* P2 purinergic receptors[9-10]. The P2 purinergic receptor, is a membrane bound receptor and can selectively combine with extracellular adenosine triphosphate to produce various biological effects. Of the purinergic receptors, the P2X receptor is a ligand-gated ion channel, and plays an important role in visceral pain[11]. Epithelial cells in tubular and saccular organs release adenosine triphosphate when affected by intense stimulation. P2X receptors then stimulate submucosal plexi to induce pain signal transduction to the brain[12-14]. P2 purinergic receptors can be divided into P2X and P2Y receptor subtypes according to their pharmacological properties[15]. P2X receptors are ligand gated, nonselective, cation channels and contain seven subtypes ($P2X_{1-7}$). These receptors are permeable to Na^+, K^+ and Ca^{2+} following activation by adenosine triphosphate. P2Y receptors are G protein coupled, and nine subtypes have been cloned from human tissues. P2X receptors are involved in the peripheral response to nociceptive and non-nociceptive stimuli. Tissue damage, visceral dilatation and sympathetic activation can induce adenosine triphosphate release, and then activate primary afferent neurons by affecting P2X receptors[16]. Adenosine triphosphate released by neurons onto primary afferent nerve terminals and secondary neurons can regulate neurotransmitter release, activate postsynaptic neurons and participate in information transfer[12, 17-18]. P2X and $P2X_3$ receptor expression are highest at the periphery[19]. At the spinal cord level, the P2X receptor on primary afferent nerve terminals can control neurotransmitter release and participate in synaptic plasticity changes in the spinal cord dorsal horn[20-21]. Recent studies have confirmed that adenosine triphosphate plays an important role in visceral pain signal transduction and visceral hyperalgesia *via* P2X and P2Y receptors, and adjusts intestinal movement and gastrointestinal secretion[22-24]. Intrathecal injection of selective $P2X_1$, $P2X_3$ and $P2X_{2/3}$ receptor antagonist trinitrophenyl adenosine triphosphate decreased visceral hypersensitivity in rats[25]. P2X receptors, especially $P2X_2$ and $P2X_3$ receptors, are involved in conduction and modulation of nociceptive information in both the peripheral and central nervous systems[12, 17].

Acupuncture and moxibustion in treatment of irritable bowel syndrome has a very long history and has been extensively used clinically[26-28]. *Tianshu* (ST25), the Front-*Mu* point of the large intestine, can regulate the intestine and *fu*-organs. *Shangjuxu* (ST37), the Lower *He*-Sea point of the large intestine, can regulate the intestine and *fu*-organs. Their combination is an effective prescription for the treatment of diarrhea and abdominal pain[29]. Recent animal studies confirmed that acupuncture and moxibustion have good therapeutic effect on enteropathies, such as ulcerative colitis[30-33] and Crohn disease[34-36]. Numerous studies showed that acupuncture induced adenosine triphosphate release in skin keratinocytes, fibroblasts and other cells, and adenosine triphosphate occupied specific receptor subtypes of skin sensory nerve endings[37-40]. Sensory nerve pulses reach the spinal cord, brainstem, hypothalamus and upper cortex *via* the ganglia. Neurons in the brainstem and hypothalamus control autonomic nerve function, including cardiovascular, gastrointestinal tract, respiratory tract, urogenital and muscle-skeletal activities. Pulses emitted by sensory nerves connected to interneurons adjusted motor neuron activity in the brainstem and hypothalamus, resulting in altering autonomic function. In particular, activated sensory neurons suppressed neural pathways in the cerebral cortex pain region *via* interneurons[37-40]. This study investigated the relationship between $P2X_2$ and $P2X_3$ receptors in dorsal root ganglia and visceral hypersensitivity. The regulatory effect of electroacupuncture at *Shangjuxu* and *Tianshu* on $P2X_2$ and $P2X_3$ receptors was also investigated to discover the mechanisms underlying electroacupuncture as a possible treatment for visceral hypersensitivity in irritable bowel syndrome.

RESULTS

Quantitative analysis of experimental animals

Twenty-four 8-day-old rats were randomly assigned to normal, model and electroacupuncture groups. A rat model of chronic visceral hypersensitivity was established in the model and electroacupuncture groups. After model establishment, rats of the electroacupuncture group received electroacupuncture bilaterally at *Shangjuxu* and *Tianshu*. All rats were included in the final analysis.

Electroacupuncture at *Shangjuxu* and *Tianshu* decreases abdominal withdrawal reflex scores in rats with visceral hypersensitivity

Abdominal withdrawal reflex scores significantly increased under colorectal distension at different intensities (2.66, 5.32, 7.98, 10.64 kPa; $P < 0.01$), indicating successful model establishment. After bilateral electroacupuncture at *Shangjuxu* and *Tianshu*, abdominal withdrawal reflex scores were significantly lower in the electroacupuncture group than in the model group ($P < 0.05$), suggesting that electroacupuncture could lessen visceral hypersensitivity in a rat model of irritable bowel syndrome.

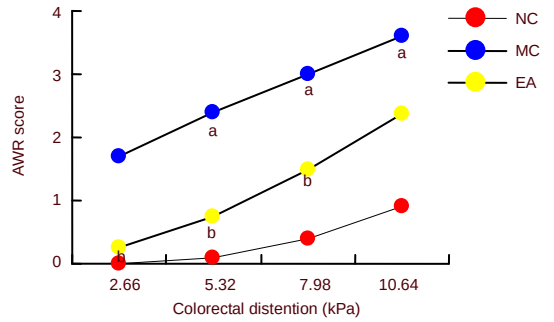

Figure 1 Effect of electroacupuncture (EA) on abdominal withdrawal reflex (AWR) scores in rats.

High AWR score represents high visceral hypersensitivity. Data are expressed as mean ± SD, eight rats in each group. One-way analysis of variance was used to compare the intergroup difference. [a]$P < 0.01$, *vs*. normal group (NC); [b]$P < 0.05$, *vs*. model group (MC).

Electroacupuncture at *Shangjuxu* and *Tianshu* diminishes $P2X_2$ receptor expression in dorsal root ganglia of rats with visceral hypersensitivity

Immunohistochemistry revealed that $P2X_2$ receptor expression increased in rat dorsal root ganglia with irritable bowel syndrome ($P < 0.01$), suggesting $P2X_2$ participated in visceral hypersensitivity. $P2X_2$ receptor expression was reduced in rat dorsal root ganglia from animals subjected to model establishment following bilateral electroacupuncture at *Shangjuxu* and *Tianshu* ($P < 0.05$), suggesting that electroacupuncture regulated visceral hypersensitivity *via* the $P2X_2$ receptor pathway (Figure 2).

Bilateral electroacupuncture at *Shangjuxu* and *Tianshu* diminishes $P2X_3$ receptor expression in dorsal root ganglion of rats with visceral hypersensitivity

Immunohistochemistry revealed that $P2X_3$ receptor expression increased in dorsal root ganglia from rats with visceral hypersensitivity ($P < 0.01$), suggesting that P2X3 participated in visceral hypersensitivity. However, $P2X_3$ receptor expression was reduced in model rats following electroacupuncture ($P < 0.05$), suggesting that electroacupuncture regulated visceral hypersensitivity using the $P2X_3$ receptor pathway (Figure 3).

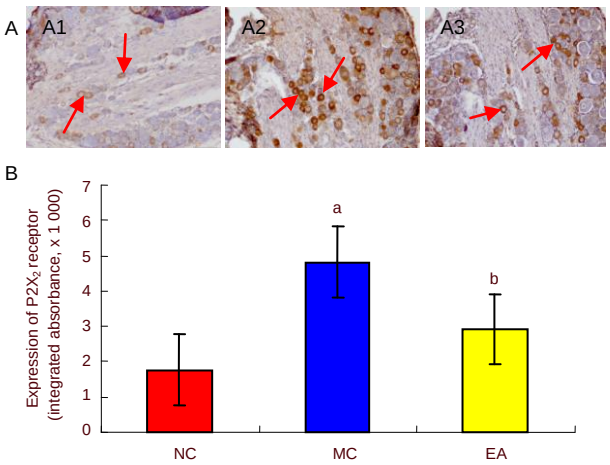

Figure 2 Effect of electroacupuncture on $P2X_2$ receptor expression in rat dorsal root ganglia.

(A) Immunohistochemistry of $P2X_2$ receptor expression in the dorsal root ganglia from experimental rats (× 200). Arrows show $P2X_2$ receptor expression. (A1–3) Normal group (NC), model group (MC) and electroacupuncture group (EA), respectively.

(B) $P2X_2$ receptor expression. Data are expressed as mean ± SD; eight rats in each group. [a]$P < 0.01$, *vs*. NC; [b]$P < 0.05$, *vs*. MC. Least significant difference *t*-test was used for intergroup comparison.

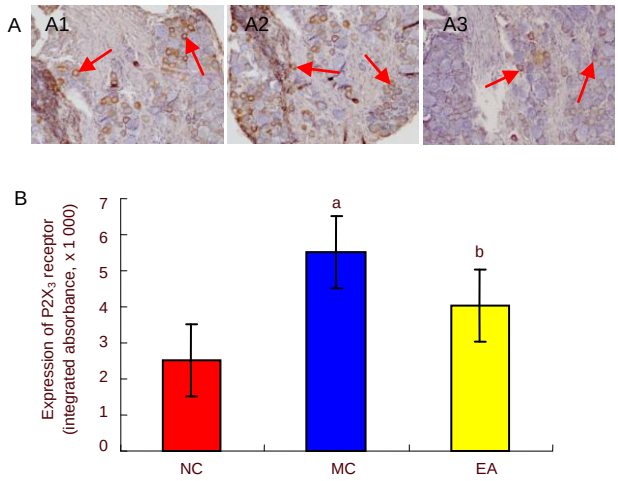

Figure 3 Effect of electroacupuncture on $P2X_3$ receptor expression in rat dorsal root ganglia.

(A) Immunohistochemistry of $P2X_3$ receptor expression in rat dorsal root ganglia (× 200). Arrows show $P2X_3$ receptor expression. (A1–3) Normal group (NC), model group (MC) and electroacupuncture group (EA), respectively.

(B) $P2X_3$ receptor expression. Data are expressed as mean ± SD; eight rats in each group. [a]$P < 0.01$, *vs*. NC; [b]$P < 0.05$, *vs*. MC. Least significant difference *t*-test was used for intergroup comparison.

DISCUSSION

A rat model of visceral hypersensitivity thought to replicate irritable bowel syndrome was established in accordance with a previously published method[41]. Neonatal rats received rectal distention, and when adult, displayed visceral hypersensitivity. Animals also showed changed stool character, which is a similar manifestation to human irritable bowel syndrome. P2X receptors are nonselective cation channels, and when activated can cause transmembrane ion influx resulting in cell depolarization. adenosine triphosphate/P2X receptors have exhibited different effects in peripheral and central mechanisms of pain[42]. $P2X_{1-6}$ receptors were visible in dorsal root ganglion, but the $P2X_3$ receptor is the most abundant. $P2X_2$ receptor expression could be detected in dorsal root ganglion, but expression was lower than that of the $P2X_3$ receptor[41], which is consistent with results from this study. P2X purinergic receptor expression is abundant in nociceptors[43]. Furthermore, cloned $P2X_3$ receptor subunit expression was only detected in sensory neurons, and only in small-diameter dorsal root ganglion cells associated with nociceptive sensations[44].

Results from the present study demonstrated that $P2X_2$ and $P2X_3$ receptors were expressed in dorsal root ganglion of rats with visceral hypersensitivity, indicating that $P2X_2$ and $P2X_3$ receptors mediated the onset of visceral hypersensitivity *via* dorsal root ganglion. Electroacupuncture could decrease visceral hypersensitivity in rats by reducing $P2X_2$ and $P2X_3$ receptor expression in dorsal root ganglion.

Numerous studies[26-28] have confirmed that acupuncture at *Shangjuxu* and *Tianshu* in the treatment of irritable bowel syndrome can obtain good outcomes. Acupuncture at *Shangjuxu* and *Tianshu* could diminish visceral hypersensitivity, and reduce abdominal withdrawal reflex scores in rat models of irritable bowel syndrome induced by colorectal stimuli[45-46]. Acupuncture at *Shangjuxu* and *Tianshu* exerts effects by regulating brain-gut peptide expression in the intestine, spinal cord and brain, such as serotonin, c-fos gene, hypothalamic adrenocorticotropin, enkephalin, endorphin and dynorphin, vasoactive intestinal peptide, substance P, prokineticin-1 and prokineticin receptor-1[45, 47-52]. Positron emission tomography revealed that electroacupuncture at *Tianshu* lessened abdominal pain, abdominal distention and abdominal discomfort by reducing glucose metabolic rate in the brain. Results from this study demonstrated that electroacupuncture at *Shangjuxu* and *Tianshu* could decrease $P2X_2$ and $P2X_3$ expression in dorsal root ganglia. This observation infers that $P2X_2$ and $P2X_3$ receptors are crucial for the effect of electroacupuncture at *Shangjuxu* and *Tianshu* in the treatment of visceral hypersensitivity in rats.

In summary, electroacupuncture at *Shangjuxu* and *Tianshu* controlled $P2X_2$ and $P2X_3$ receptor expression in dorsal root ganglia of rats subjected to a model of irritable bowel syndrome. This acupuncture also contributed to the improvement of visceral hypersensitivity induced by this model. These findings may illustrate the effectiveness of electroacupuncture for the treatment of irritable bowel syndrome.

MATERIALS AND METHODS

Design

Randomized, controlled animal study.

Time and setting

Experiments were performed from May to September 2011. Model establishment, treatment, and sample collection were conducted at the Experimental Animal Department, Shanghai Medical College, Fudan University, China. Index detection was conducted at the Three-Level Laboratory of Acupuncture and Immunity, State Administration of Traditional Chinese Medicine.

Materials

A total of 24, specific pathogen free, male, neonatal, Sprague-Dawley rats aged 8 days were supplied by the Experimental Animal Department, Shanghai Medical College, Fudan University, China (animal license No. SCXK (Hu) 2009-0019). Eight neonatal rats and a female suckling rat were housed in a plastic cage with a 12-hour light/dark cycle at 22 ± 2°C and humidity of 50–70%. The suckling rat was allowed free access to water and food. All protocols were in accordance with *Guidance Suggestions for the Care and Use of Laboratory Animals*, formulated by the Ministry of Science and Technology of China[53].

Methods

Establishment of a rat model of visceral hypersensitivity mimicking irritable bowel syndrome

A rat model for visceral hypersensitivity in irritable bowel syndrome were induced according to a previously published method[53]. The self-made saccule coated with vaseline was slowly inserted into the descending colon

via the anus to a depth of about 2 cm. Saccule distension (0.2 mL) occurred for 1 minute. One hour later, the same stimulus was repeated once for 2 minutes. Model induction lasted for 14 consecutive days. Stool character was observed and abdominal withdrawal reflex was scored to evaluate the sensitivity of intrarectal distension in rats and to identify whether model establishment was successful. Rats were housed for 6–8 weeks. Rats in the normal group only underwent handling, gripping and massaging in the perineal region.

Electroacupuncture stimuli

The day after model induction, electroacupuncture was conducted using a Han's acupoint nerve stimulator (Nanjing Gensun Technology, Nanjing, Jiangsu Province, China) at *Tianshu* (0.2 cm lateral to the intersection of upper 8/13 and lower 5/13 from xiphoid to symphysis) and *Shangjuxu* (intersection of upper 6/16 and lower 10/16 of lateral condyle of tibia and lateral malleolus, 0.1 cm lateral to crista anterior tibiae)[54] for 20 minutes. The depth of needling was 5 mm. The frequency of sparse and dense waves was 2–100 Hz, with a current of 2 mA, once a day, for 7 consecutive days. Within 60 minutes after the last electroacupuncture, colorectal distension was performed. Simultaneously, abdominal withdrawal reflex was observed to evaluate the effect of electroacupuncture in each group. Stimulus method: the self-made saccule was connected to a T-valve. One end was connected to a 10-mL syringe and the other end was connected to a blood pressure monitor (Shanghai Medical Instruments Co., Ltd., Shanghai, China). After the saccule was inserted, stimuli at 2.66, 5.32, 7.98, or 10.64 kPa were given. Each stimulus lasted for about 20 seconds with a 5-minute interval. Each stimulus intensity was repeated three times, and the average was calculated. Reactions were scored in accordance with a previous method[55]. Scores were used as follows: 0 score: no behavior reaction; 1: movement standstill and transient head movement; 2: contraction of abdominal muscles during stimuli; 3: raised abdomen; 4: body raised, elevation of pelvic cavity and scrotum.

Sample collection of dorsal root ganglion

After abdominal withdrawal reflex scoring, rats were intraperitoneally anesthetized with 10% chloral hydrate. Saline (100 mL) was rapidly perfused into the ascending aorta through left ventricle intubation, followed by 4% paraformaldehyde (300 mL) at 4°C. Bilateral dorsal root ganglia at L_6–S_3 were dissociated and collected under an anatomical microscope (SZX10; Olympus, Tokyo, Japan). Tissue was dehydrated, embedded in paraffin, and then sliced into 5-μm thick sections.

Immunohistochemistry for $P2X_2$ and $P2X_3$ receptor expression in rat dorsal root ganglia

After dewaxing, antigen was retrieved by microwave heating. Tissue was incubated in mouse anti-rat $P2X_2$ and $P2X_3$ monoclonal antibody (1:400; Alomone Labs, Israel) at 37°C for 2 hours, and then washed in 0.01 M PBS (pH 7.2–7.6) five times, at 5 minutes each. Using a goat anti-rabbit/rat immunohistochemistry kit [Gene Tech (Shanghai) Co., Ltd., Shanghai, China], tissues were incubated with the A liquid in a wet box for 30 minutes at room temperature, immersed in 0.01 M PBS three times (5 minutes), followed by development in 3,3'-diaminobenzidine. Staining was terminated using PBS (0.01 M) or distilled water under a light microscope (Olympus). Tissue was stained with hematoxylin, treated with 1% acidic alcohol for color separation, dehydrated, permeabilized, and then mounted with neutral gum. PBS was used as a negative control. Images were collected from three fields randomly selected under a 400 × light microscope. Mean value of integral absorbance was calculated using Motic Med 6.0 image analysis (Beijing Maikeaodi Image Technique Co., Ltd., Beijing, China).

Statistical analysis

All data were analyzed using SPSS 18.0 software (SPSS Inc., Chicago, IL, USA), and were expressed as mean ± SD. One-way analysis of variance was used for intergroup comparison. Least significant difference *t*-test was utilized for paired comparison among multiple means. A value of $P < 0.05$ was considered statistically significant.

Acknowledgments: We thank associate researchers Yunhua Cui and Xiaomei Wang from Shanghai Academy of Traditional Chinese Medicine in China for drafting the manuscript.

Funding: This project was funded by the National Natural Science Foundation of China, No. 30973783; the Shanghai Municipal Scientific Committee Project, No. 11ZR1434300; and the Key Subject Program of State Administration of Traditional Chinese Medicine.

Author contributions: Yuhu Xin participated in study concept and design. Zhijun Weng, Yuan Lu, Lidong Wang and Ming Dong performed model establishment, collected samples and detected indexes. Zhijun Weng and Luyi Wu performed data analysis and wrote the manuscript. Yuhu Xin and Linying Tan provided technical and data support. All authors have read and agreed to the manuscript as written.

Conflicts of interest: None declared.

Ethical approval: This study was approved by the Animal Ethics Committee, Yueyang Hospital of Integrated Traditional Chinese and Western Medicine, Shanghai University of Traditional Chinese Medicine in China.

Author statements: The manuscript is original, has not been submitted to or is not under consideration by another publication, has not been previously published in any language or any form, including electronic, and contains no disclosure of confidential information or authorship/patent application/funding source disputations.

REFERENCES

[1] Kanazawa M, Hongo M, Fukudo S. Visceral hypersensitivity in irritable bowel syndrome. J Gastroenterol Hepatol. 2011;26 Suppl 3:119-121.
[2] Keszthelyi D, Troost FJ, Masclee AA. Irritable Bowel Syndrome: Methods, Mechanisms, and Pathophysiology. Methods to assess visceral hypersensitivity in irritable bowel syndrome. Am J Physiol Gastrointest Liver Physiol. 2012;303(2):G141-154.
[3] Mathew P, Bhatia SJ. Pathogenesis and management of irritable bowel syndrome. Trop Gastroenterol. 2009;30(1): 19-25.
[4] Kang MX, Jia H. Progress in mechanisms of visceral hypersensitivity in irritable bowel syndrome. Shijie Huaren Xiaohua Zazhi. 2008;16(14):1554-1558.
[5] Elsenbruch S. Abdominal pain in Irritable Bowel Syndrome: a review of putative psychological, neural and neuro-immune mechanisms. Brain Behav Immun. 2011;25(3):386-394.
[6] Grundy D. Speculations on the structure/function relationship for vagal and splanchnic afferent endings supplying the gastrointestinal tract. J Auton Nerv Syst. 1988;22(3):175-180.
[7] Furness JB, Clerc N, Kunze WA. Memory in the enteric nervous system. Gut. 2000;47 Suppl 4:iv60-62;iv76.
[8] Szurszewski JH, Ermilov LG, Miller SM. Prevertebral ganglia and intestinofugal afferent neurones. Gut. 2002;51 Suppl 1:i6-10.
[9] Burnstock G. Introductory overview of purinergic signalling. Front Biosci (Elite Ed). 2011;3:896-900.
[10] Burnstock G. The past, present and future of purine nucleotides as signalling molecules. Neuropharmacology. 1997;36(9):1127-1139.
[11] Shieh CC, Jarvis MF, Lee CH, et al. P2X receptor ligands and pain. Expert Opin Ther Pat. 2006;16(8):1113-1127.
[12] Burnstock G. Purinergic P2 receptors as targets for novel analgesics. Pharmacol Ther. 2006;110(3):433-454.
[13] Yu Q, Zhao Z, Sun J, et al. Expression of P2X6 receptors in the enteric nervous system of the rat gastrointestinal tract. Histochem Cell Biol. 2010;133(2):177-188.
[14] Xu GY, Huang LY. Peripheral inflammation sensitizes P2X receptor-mediated responses in rat dorsal root ganglion neurons. J Neurosci. 2002;22(1):93-102.
[15] Khakh BS, Burnstock G, Kennedy C, et al. International union of pharmacology. XXIV. Current status of the nomenclature and properties of P2X receptors and their subunits. Pharmacol Rev. 2001;53(1):107-118.
[16] Burnstock G. Purinergic mechanosensory transduction and visceral pain. Mol Pain. 2009;5:69.
[17] Burnstock G. Purine-mediated signalling in pain and visceral perception. Trends Pharmacol Sci. 2001;22(4): 182-188.
[18] Burnstock G. Targeting the visceral purinergic system for pain control. Curr Opin Pharmacol. 2012;12(1):80-86.
[19] Dunn PM, Zhong Y, Burnstock G. P2X receptors in peripheral neurons. Prog Neurobiol. 2001;65(2): 107-134.
[20] Ruan HZ, Burnstock G. Localisation of P2Y1 and P2Y4 receptors in dorsal root, nodose and trigeminal ganglia of the rat. Histochem Cell Biol. 2003;120(5):415-426.
[21] Lin SY, Corey DP. TRP channels in mechanosensation. Curr Opin Neurobiol. 2005;15(3):350-357.
[22] Xu GY, Shenoy M, Winston JH, et al. P2X receptor-mediated visceral hyperalgesia in a rat model of chronic visceral hypersensitivity. Gut. 2008;57(9):1230-1237.
[23] Cooke HJ. Neuroimmune signaling in regulation of intestinal ion transport. Am J Physiol. 1994;266(2 Pt 1): G167-178.
[24] Burnstock G. Purines and sensory nerves. Handb Exp Pharmacol. 2009;(194):333-392.
[25] Cao HL, Gao J, Jin ZD, et al. Effect of intrathecal injection of trinitrophenyl adenosine triphosphate on visceral hypersensitivity in rats. Wei Chang Bing Xue. 2007;12(6): 344-348.
[26] Manheimer E, Wieland LS, Cheng K, et al. Acupuncture for irritable bowel syndrome: systematic review and meta-analysis. Am J Gastroenterol. 2012;107(6): 835-848.
[27] Manheimer E, Cheng K, Wieland LS, et al. Acupuncture for treatment of irritable bowel syndrome. Cochrane Database Syst Rev. 2012;5:CD005111.
[28] Zhu WL, Li Y, Wei HF, et al. Effect of electro-acupuncture at different acupoints on neuropeptide and somatostatin in rat brain with irritable bowel syndrome. Chin J Integr Med. 2012;18(4):288-292.
[29] Zhang HS, Wang FC. Application of combination of he-mu points and combination of shu-yuan in syndrome differentiation of zang- and fu-organs. Zhongguo Zhenjiu. 2006;26(5):378-380.
[30] Wu HG, Zhou LB, Pan YY, et al. Study of the mechanisms of acupuncture and moxibustion treatment for ulcerative colitis rats in view of the gene expression of cytokines. World J Gastroenterol. 1999;5(6):515-517.
[31] Wu HG, Zhou LB, Shi DR, et al. Morphological study on colonic pathology in ulcerative colitis treated by moxibustion. World J Gastroenterol. 2000;6(6):861-865.
[32] Wu HG, Gong X, Yao LQ, et al. Mechanisms of acupuncture and moxibustion in regulation of epithelial cell apoptosis in rat ulcerative colitis. World J Gastroenterol. 2004;10(5):682-688.
[33] Wu HG, Liu HR, Tan LY, et al. Electroacupuncture and moxibustion promote neutrophil apoptosis and improve ulcerative colitis in rats. Dig Dis Sci. 2007;52(2):379-384.

[34] Shi Y, Zhou EH, Wu HG, et al. Moxibustion treatment restoring the intestinal epithelium barrier in rats with Crohn's disease by down-regulating tumor necrosis factor alpha, tumor necrosis factor receptor 1, and tumor necrosis factor receptor 2. Chin J Integr Med. 2011;17(3): 212-217.
[35] Bao CH, Wu LY, Shi Y, et al. Moxibustion down-regulates colonic epithelial cell apoptosis and repairs tight junctions in rats with Crohn's disease. World J Gastroenterol. 2011; 17(45):4960-4970.
[36] Bao CH, Wu LY, Wu HG, et al. Moxibustion inhibits apoptosis and tumor necrosis factor-alpha/tumor necrosis factor receptor 1 in the colonic epithelium of Crohn's disease model rats. Dig Dis Sci. 2012;57(9):2286-2295.
[37] Burnstock G. Acupuncture: a novel hypothesis for the involvement of purinergic signalling. Med Hypotheses. 2009;73(4):470-472.
[38] Gourine AV, Wood JD, Burnstock G. Purinergic signalling in autonomic control. Trends Neurosci. 2009;32(5): 241-248.
[39] Zhao ZQ. Neural mechanism underlying acupuncture analgesia. Prog Neurobiol. 2008;85(4):355-375.
[40] Yu XJ, Ding GH, Yao W, et al. The role of collagen fiber in "Zusanli" (ST 36) in acupuncture analgesia in the rat. Zhongguo Zhen Jiu. 2008;28(3):207-213.
[41] Labrakakis C, Gerstner E, MacDermott AB. Adenosine triphosphate-evoked currents in cultured dorsal root ganglion neurons obtained from rat embryos: desensitization kinetics and modulation of glutamate release. Neuroscience. 2000;101(4):1117-1126.
[42] Hamilton SG, McMahon SB. ATP as a peripheral mediator of pain. J Auton Nerv Syst. 2000;81(1-3):187-194
[43] Dowd E, McQueen DS, Chessell IP, et al. P2X receptor-mediated excitation of nociceptive afferents in the normal and arthritic rat knee joint. Br J Pharmacol. 1998;125(2): 341-346.
[44] Chen CC, Akopian AN, Sivilotti L, et al. A P2X purinoceptor expressed by a subset of sensory neurons. Nature. 1995;377(6548):428-431.
[45] Wu HG, Jiang B, Zhou EH, et al. Regulatory mechanism of electroacupuncture in irritable bowel syndrome: preventing MC activation and decreasing SP VIP secretion. Dig Dis Sci. 2008;53(6):1644-1651.
[46] Liu HR, Wang XM, Zhou EH, et al. Acupuncture at both ST25 and ST37 improves the pain threshold of chronic visceral hypersensitivity rats. Neurochem Res. 2009; 34(11):1914-1918.
[47] Zhou EH, Liu HR, Wu HG, et al. Suspended moxibustion relieves chronic visceral hyperalgesia via serotonin pathway in the colon. Neurosci Lett. 2009;451(2):144-147.
[48] Zhou EH, Liu HR, Wu HG, et al. Herb-partition moxibustion relieves chronic visceral hyperalgesia and 5-HT concentration in colon mucosa of rats. Neurol Res. 2009;31(7):734-737.
[49] Zhou EH, Wang XM, Ding GH, et al. Suspended moxibustion relieves chronic visceral hyperalgesia and decreases hypothalamic corticotropin-releasing hormone levels. World J Gastroenterol. 2011;17(5):662-665.
[50] Ma XP, Tan LY, Yang Y, et al. Effect of electro-acupuncture on substance P, its receptor and corticotropin-releasing hormone in rats with irritable bowel syndrome. World J Gastroenterol. 2009;15(41):5211-5217.
[51] Wu HG, Liu HR, Zhang ZA, et al. Electro-acupuncture relieves visceral sensitivity and decreases hypothalamic corticotropin-releasing hormone levels in a rat model of irritable bowel syndrome. Neurosci Lett. 2009;465(3): 235-237.
[52] Liu HR, Qi L, Wu LY, et al. Effects of moxibustion on dynorphin and endomorphin in rats with chronic visceral hyperalgesia. World J Gastroenterol. 2010;16(32): 4079-4083.
[53] The Ministry of Science and Technology of the People's Republic of China. Guidance Suggestions for the Care and Use of Laboratory Animals. 2006-09-30.
[54] Li ZR. Experimental Acupuncture. Beijing: China Press of Traditional Chinese Medicine. 2007.
[55] 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(5):1276-1285.

(Edited by Song XG, Wu XG/Qiu Y/Wang L)