



Repeated transcutaneous electrical nerve stimulation of nonspecific acupoints of the upper body attenuates stress-induced visceral hypersensitivity in rats

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ARTICLE INFO

Keywords:

Irritable bowel syndrome
Stress
Visceral hypersensitivity
Transcutaneous electrical nerve stimulation
Acupoint specificity

ABSTRACT

Irritable bowel syndrome (IBS) is a common stress-related gastrointestinal disorder and visceral hypersensitivity (VH) is characteristically found in IBS patients. Transcutaneous electrical nerve stimulation (TENS) applied to certain acupoints has been shown to benefit IBS patients. Here, we investigated whether nonspecific acupoint is involved in the efficacy of TENS treatment for IBS. Twenty-five male rats were randomly assigned to four experimental groups and one sham-control group. The four experimental groups were defined as TENS-RR, TENS-RL, TENS-LR, and TENS-LL based on the location of the two TENS patches [right (R) or left (L)]. The former and latter letter pairs indicate that the patch locations were the upper chest and upper back, respectively. The heterotypic intermittent stress (HIS) protocol was performed for 16 days. VH was assessed by electromyography to evaluate response to rectal distention (RD). Modulated medium-frequency TENS, sweep range 1–10 Hz, amplitude slightly above the supra motor threshold, was applied 30 min per day followed by RD every second day for the final 7 days of the 16-day HIS period. VH was induced after the rats had been subjected to HIS for 10 days. A significant reduction of VH was observed only in the TENS-LL group compared with that in the sham-control group. These data suggest that repeated TENS treatment can alleviate stress-induced VH in rats. Further, whether TENS patches are attached to the left or right side of the body, which are nonspecific acupoints for gastrointestinal functions, may be an important factor in the treatment of stress-associated gastrointestinal symptoms.

1. Introduction

Irritable bowel syndrome (IBS) is a common gastrointestinal disorder that typically manifests as chronic visceral pain or abdominal discomfort accompanied by disturbances in bowel movements, including constipation, diarrhea, or both (Mayer and Gebhart, 1994; Bueno et al., 1997; Al-Chaer et al., 2000). Although IBS is not life-threatening, it can negatively impact health-related quality of life. No apparent structural or biochemical abnormalities can completely explain IBS symptoms (Drossman et al., 2002), and its etiological factors remain unclear. Growing evidence suggests that visceral hypersensitivity (VH), a biological marker of IBS, plays an important role in the pathophysiology of IBS (Mertz et al., 1995; Bouin et al., 2002; Delvaux, 2002; Kuiken et al., 2005; Azpiroz et al., 2007; Piche et al., 2010; Ludidi et al., 2012). Visceral hypersensitivity is associated with abnormal

processing of visceral nociceptive signals in the brain-gut axis (Mertz, 2002) and is recognized as an important characteristic of IBS patients (Delvaux, 2002).

Since its discovery in the early 1950s, transcutaneous electrical nerve stimulation (TENS) has been used to treat pain, musculoskeletal conditions (Johnson and Tabasam, 2003; Ward and Chuen, 2009), urinary incontinence (Dumoulin et al., 1995; Kajbafzadeh et al., 2009), and wounds (Ganne, 1988). In recent years, TENS has become increasingly popular for treating gastrointestinal disorders (Furgała et al., 2001; Xiao and Liu, 2004; King et al., 2004; Eléouet et al., 2010; Leroi et al., 2012; McNearney et al., 2013; Bouguen et al., 2014; Zhang et al., 2015). A significant increase in the percentage of normal frequency and a decrease in tachygastric rhythm of gastric myoelectrical activity has been demonstrated by cutaneous electrical stimulation at acupoint ST36 (Zusanli) in healthy volunteers (Chang et al., 2002). Short-term

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TENS at acupoints ST36, LI4 (Hegu), and UB57 (Chenshan) has been shown to increase rectal sensory thresholds in patients with diarrhea-predominant IBS. In these patients, consistent TENS treatment for 2 months resulted in a significant increase in rectal sensory thresholds and a decrease in stool times and intensity of abdominal pain (Xiao and Liu, 2004). Moreover, TENS at ST36 and PC6 (Neiguan), but not sham stimulation, significantly increased the threshold of rectal sensation of gas, desire to defecate, and pain in IBS patients compared to a control period (Xing et al., 2004). Gastric hypomotility and the association between gastric myoelectrical activity and sympathovagal balance are ameliorated by daily TENS treatment at acupoints ST36 and PC6 for 2 weeks in scleroderma patients (McNearney et al., 2013). In addition, Zhang et al. (2015) reported that TENS at acupoint ST36 improved cold stress-induced impairment in gastric slow waves in healthy volunteers. Thus, application of TENS to specific acupoints related to gastrointestinal functions may serve an important therapeutic role in patients with gastrointestinal disorders. However, it is unclear whether acupoint specificity is crucial for the treatment of IBS by TENS. Thus, in the present study, we assessed the effects of TENS patches attached to the left or right upper chest and back, which are nonspecific acupoints, on gastrointestinal function in rats.

Stress is known to play a major role in the development of IBS (Konturek et al., 2011). Heterotypic intermittent stress (HIS) consists of both physical and psychological stressors and is used to study stress-induced VH in animals (Winston et al., 2010; Wang et al., 2012; Zhou et al., 2012; Zhang et al., 2014). Rats subjected to HIS exhibit no injury or robust inflammation in the colon, but do show noticeable visceromotor responses to colorectal distention compared with controls. This animal model resembles some characteristics of IBS patients. Therefore, the aim of the present study was to investigate whether repeated TENS treatment at acupoints not specifically related to gastrointestinal functions could have therapeutic effects on HIS-induced VH in rats.

2. Materials and methods

2.1. Animals

All experiments were performed with adult male Sprague-Dawley rats weighing 250–300 g (BioLASCO Animal Center, Taipei, Taiwan). Animals were housed in a temperature-controlled room ($24^{\circ}\text{C} \pm 2^{\circ}\text{C}$) on a 12 h/12 h light/dark cycle (lights on at 8:00 h). Food pellets and water were available ad libitum. All experimental procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of the College of Medicine, National Taiwan University (IACUC approval number 20170011). All rats were subjected to HIS and then randomly divided into four equal experimental groups and one sham-control group. The experimental groups received TENS whereas the sham-control group received identical TENS treatment without the delivery of electrical currents to the patch electrodes. The four experimental groups were designated TENS-RR, TENS-RL, TENS-LR, and TENS-LL according to the four different combinations of patch locations that are nonspecific acupoints for gastrointestinal function. The letter R and L indicates that the TENS patches were attached to the right or left side of the upper body, respectively. Moreover, the first letter of the letters pair indicates that the patch location was the upper chest, while the second letter indicates that the patch location was the upper back. All efforts were made to minimize suffering and the number of animals used. There was no alternative to the use of whole animals.

2.2. Electrode implantation for electromyographic recording

After arrival at the laboratory, all rats were allowed to rest for one week. After overnight fasting, rats were anesthetized by intraperitoneal injection of sodium pentobarbital (50 mg/kg, P3761, Sigma-Aldrich, St.

Louis, MO, USA) for the implantation of three perfluoroalkoxy-coated stainless-steel wires (793,200, A-M Systems, Sequim, WA, USA) to the left external oblique muscle, 1 cm above the inguinal ligament. Three electrode wires, stitched in parallel (1 cm apart), were tunneled subcutaneously and externalized in the region of the dorsal scapula. One electrode was used for grounding and the other two electrodes were used for electromyographic (EMG) recordings. After abdominal electrode implantation, each rat was housed individually for one week for recovery.

2.3. Heterotypic intermittent stress protocol

To induce VH, a HIS protocol comprising three stressors in a random sequence—cold restraint stress, water avoidance stress, and forced swimming stress—was performed. This stress protocol was slightly modified from that used by Winston et al. (2010). Our HIS protocol was performed for 16 consecutive days. Each day, rats were subjected to one of the three stressors between 9:00 h and 12:00 h. Each stressor was administered 5–6 times per rat for 16 consecutive days, with the same stressor not used on consecutive days. For cold restraint stress, rats were restrained in a plastic container (6 cm diameter, 18 cm length) with openings (2 cm diameter) at each end. The container was then placed in a 4°C room for 45 min. For water avoidance stress, rats were placed on a cylinder (8 cm diameter, 20 cm height) in a bucket (47 cm diameter, 59 cm height) filled with 15°C water to a depth within 1 cm of the top of the cylinder at room temperature (approximately 25°C) for 60 min. For forced swimming stress, rats were forced to swim in a bucket (47 cm diameter, 59 cm height) filled with 25°C water at a depth of 11–13 cm for 20 min at room temperature (approximately 25°C).

2.4. Transcutaneous electrical nerve stimulation procedure

Under isoflurane anesthesia (G-8669, Panion & BF Biotech, Taipei, Taiwan), upper body of the rats was shaved and coated with a conductive gel to allow the electrode patch to make good contact with the skin. Two rectangular ($1.4\text{ cm} \times 2.5\text{ cm}$) self-adhesive electrode patches were attached to the upper chest and back on the left or right side. For example, an electrode patch was affixed to the left upper back along the entire scapula (Fig. 1A), and the other electrode patch was placed between the left clavicle and left armpit (Fig. 1B) in the TENS-LL group. Rats were then wrapped with an elastic mesh bandage ($2.5\text{ cm} \times 8\text{ cm}$) from the neck to the chest to secure the two patches (Fig. 1C). Both electrode patches were connected to a commercial constant-current TENS stimulator (Powered Muscle Stimulator- Multiple Middle Frequency Stimulator AMSTIM 572, Ever Prosperous Instrument, New Taipei City, Taiwan). The carrier frequency was 5-kHz sine wave. The modulation frequency showed a 50% duty cycle square wave, with an up–down sweep from 1 to 10 Hz and back to 1 Hz once per second. The electrical stimulation intensity was adjusted to slightly exceed the motor threshold based on visual feedback, which was generally reached at amplitudes of roughly 6 mA peak-to-peak. This intensity remained unchanged over the application period of 30 min. During TENS, the rats were awake and allowed to move freely. The rats did not show any uncomfortable reactions during TENS stimulation.

2.5. Measurement of visceromotor response

After an overnight fast and the termination of TENS treatment, rats were lightly anesthetized with isoflurane. The rat's anus was gently massaged to clean stool from the rectum, and a 6-Fr/CH Silicon balloon-urethral catheter (1821-0506, Fortune Medical Instrument, New Taipei City, Taiwan) was then inserted intra-anally 4.6 cm into the rectum. The catheter was fixed to the rat's tail with medical tape to prevent displacement. The animal was then placed in a rodent restrainer (20 cm length, 7 cm outer diameter, 6 cm inner diameter; STM-

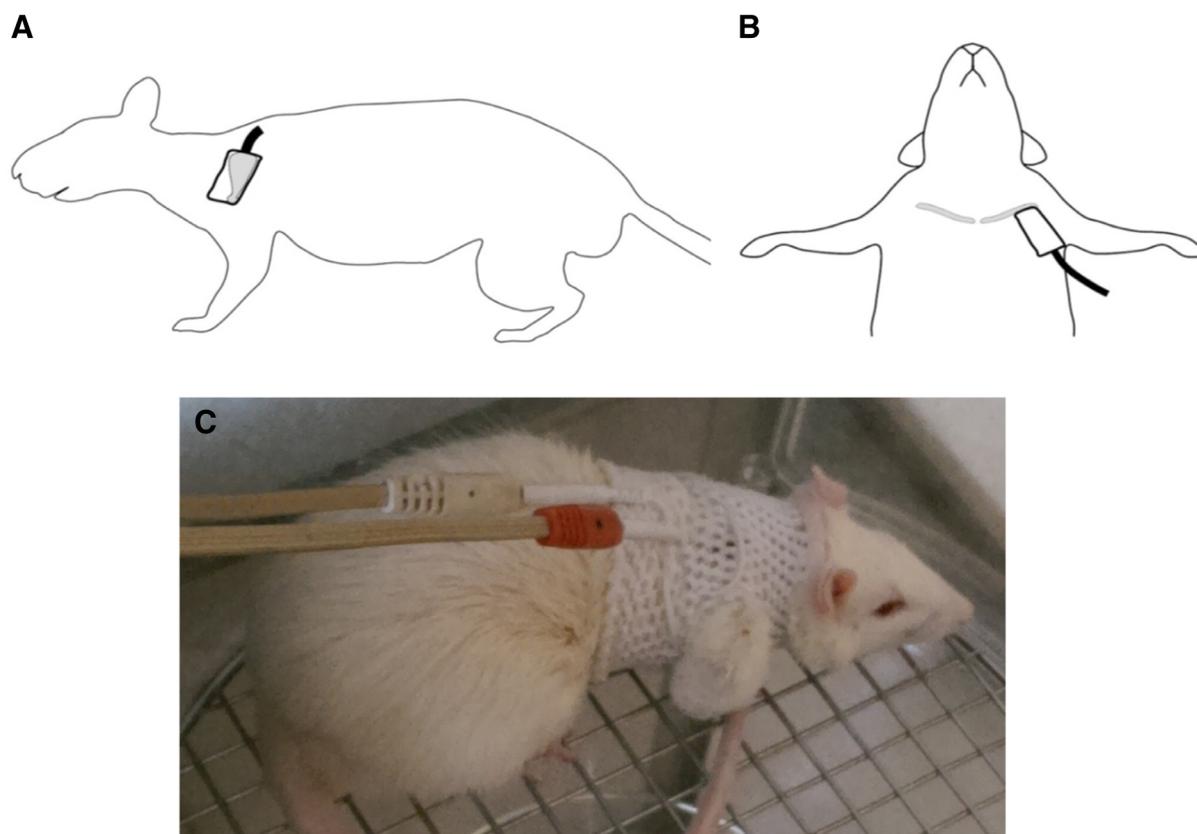


Fig. 1. Schematic drawings of the locations of TENS patches on the back and chest of rats. Two rectangular self-adhesive electrode patches (1.4 cm × 2.5 cm) were separately attached to the left or right side of the body surface, one on the upper back and one on the chest. One patch was affixed to the left upper back along the entire scapula (A), and the other patch was placed between the left clavicle and left armpit (B). The upper body from the neck to the chest was covered with an elastic mesh bandage (2.5 cm × 8 cm) to secure the two TENS patches (C).

6, Shineteh Instruments, Taipei, Taiwan). Three electrodes were connected to a Biopac System (MP30, Biopac Systems, Goleta, CA, USA) to collect EMG signals from abdominal external oblique muscle contractions induced by graded rectal distention (RD). The rats were allowed to fully awaken from anesthesia and then acclimatized for 30 min in the restrainer. The RD procedure was slightly modified from a previous report (Shamshiri et al., 2009). The distention cycle comprised rapid phasic distention of five different volumes (0.2, 0.4, 0.6, 0.8, and 1 ml water), each applied for a 20 s stimulation period followed by a 2 min rest interval. This procedure was repeated three times at 5 min intervals for each rat. The balloon was distended by quickly injecting the required volume of pre-warmed (37 °C) water, followed by complete removal of the water with a syringe at the end of each distention to ensure that the balloon did not leak. EMG signals from abdominal striated muscles were recorded, which were then amplified and digitized using Biopac Student Lab 3.7.7 software (Biopac Systems, Goleta, CA, USA). The area-under-the-curve (AUC), expressed as the integral ($\mu\text{V} \times \text{seconds}$) of the EMG signals, was calculated by an internal program. The net value for each distention period was calculated by subtracting the baseline value derived from the AUC 10 s before and 10 s after distending the balloon. Triplicate EMG data were obtained from each rat at 5 min intervals between distention cycles. The collection of EMG recordings and analysis of AUC data were performed by different researchers.

2.6. Experimental procedures

No significant differences in EMG activity were seen between the sham-control and experimental TENS groups on Day 0, indicating that baseline EMG activity was the same in all animals. As shown in Fig. 2,

HIS was administered between 9:00 h and 12:00 h every day from Day 1 to Day 16 to induce VH of the IBS model. Since the sham-control had significantly higher EMG activity induced by 10-day HIS on Day 10 compared with baseline, TENS treatment was performed from 12:30 h to 13:30 h every day from Day 10 to Day 16 (7 days). RD was conducted after TENS treatment on every second day from Day 10 to Day 16 (i.e., Day 10, Day 12, Day 14, and Day 16). The sham-control received identical TENS treatment without electrical stimulation from Day 10 to Day 16 (7 days).

2.7. Sample size and statistical analysis

In the original experimental design, we planned to assign seven rats to each group. However, due to the death of animals, imperfect treatment procedures, and abnormal experimental values from some animals, for the final data analysis there were five animals in each group. We used the Smirnov-Grubbs test to assess anomalous values in each group at the 0.05 significance level. Power analysis showed an observed power of 0.848, indicating that the number of animals per group was appropriate.

Data were analyzed using SPSS 13.0 software (IBM, Armonk, NY, USA). All data are expressed as mean \pm standard error of the mean. One-way repeated-measures analysis of variance followed by Fisher's Least Significant Difference post hoc test was used to compare the AUCs of EMG data. p -Values < 0.05 were considered statistically significant.

3. Results

There were no significant changes in EMG activity in any of the TENS groups compared with the sham-control on Day 10, indicating

Day 0	Day 1	8 days	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16
	HIS	HIS	HIS	HIS	HIS	HIS	HIS	HIS	HIS
Baseline			T/S						
			RD		RD		RD		RD

Fig. 2. Experimental design. After recovery from wire electrode implantation, the rectal distention (RD) procedure was performed on Day 0 to collect baseline EMG recordings. Heterotypic intermittent stress (HIS) was administered from 9:00 h to 12:00 h daily from Day 1 to Day 16 to induce visceral hypersensitivity of the IBS model. TENS treatment (T) was performed from 12:30 h to 13:30 h daily from Day 10 to Day 16 (7 days). Rectal distention was performed after TENS treatment on Days 10, 12, 14, and 16. Rats in the sham-control group received identical TENS treatments without electrical current delivery to the patch electrodes (S) for seven days.

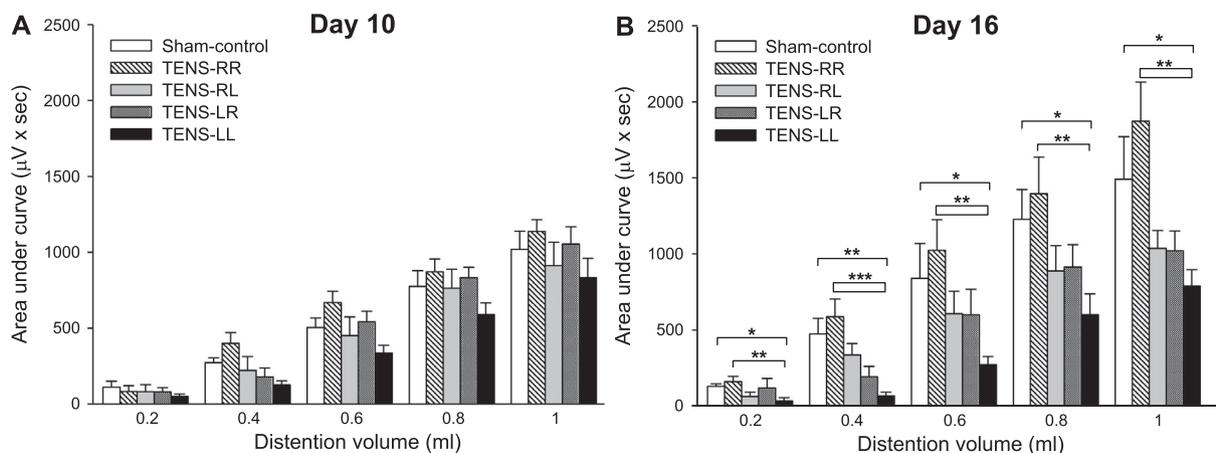


Fig. 3. Comparison of EMG activity induced by the first TENS treatment on Day 10 and the 7th treatment on Day 16 in the TENS (TENS-RR, TENS-RL, TENS-LR, and TENS-LL) and sham-control groups. The letters R and L indicate that TENS patches were attached to the right or left side of the upper body, respectively. The first letter of the letter pair indicates that the patch location was the upper chest, while the second letter indicates that the patch location was the upper back. On Day 10, the first TENS treatment did not result in significant differences in EMG activity between any of the TENS groups and the sham-control (A). On Day 16, EMG activity in the TENS-LL group after 7 TENS treatments was significantly lower than that in the sham-control and TENS-RR groups at each distention volume (B). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

that the first TENS treatment did not alter EMG activity on Day 10 in any group (Fig. 3A). By contrast, repeated TENS treatment significantly reduced EMG activity in the TENS-LL group compared with the sham-control ($p = 0.012$) at distention volumes of 0.2 ml ($p = 0.035$), 0.4 ml ($p = 0.002$), 0.6 ml ($p = 0.029$), 0.8 ml ($p = 0.024$), and 1 ml ($p = 0.019$) on Day 16 (Fig. 3B). These data suggest that EMG activity can be significantly suppressed by repeated TENS treatment in TENS-LL, but not in the other TENS groups. In addition, EMG activity in the TENS-LL group after seven TENS treatments was significantly lower than that in the TENS-RR group at distention volumes of 0.2 ml ($p = 0.007$), 0.4 ml ($p = 0.001$), 0.6 ml ($p = 0.006$), 0.8 ml ($p = 0.006$), and 1 ml ($p = 0.001$) on Day 16 (Fig. 3B). These results suggest that the effects of TENS treatment may be associated with the left or right side of the body where the TENS patches are attached.

4. Discussion

The present study demonstrated the effects of repeated TENS treatment to the upper body at acupoints nonspecific to gastrointestinal functions on HIS-induced VH in rats. Although TENS is used to alleviate various types of pain, few scientific validations of using TENS for the treatment of visceral pain have been published (Börjesson et al., 1998; Iovino et al., 2006; Leung et al., 2013). Here, we showed that repeated TENS treatment significantly reduced EMG activity (Fig. 3B) in response to RD in rats subjected to HIS, indicating that TENS treatment at nonspecific acupoints had an analgesic effect. Further, our results provide evidence that repeated TENS treatment applied at acupoints unrelated to gastrointestinal functions attenuates stress-induced VH in rats, and that the length of treatment as well as the attachment sites of

the TENS patches are critical. These findings are consistent with a previous report in which long-term TENS treatment exerted stronger therapeutic effects in patients with diarrhea-predominant IBS (Xiao and Liu, 2004).

The fundamental concept of the therapeutic effects of acupuncture, electroacupuncture (EA), and acupoint TENS involves the stimulation of specific acupoints. Therefore, the selection of specific acupoints is crucial for producing the desired therapeutic outcomes. Although some studies have reported improvement in bowel symptoms in IBS patients treated with acupuncture (Chan et al., 1997; Chao and Zhang, 2014; Zhang et al., 2018) or TENS (Xiao and Liu, 2004; Xing et al., 2004), other studies suggest that any benefits are placebo effects (Rohrböck et al., 2004; Schneider et al., 2006; Lembo et al., 2009; Lowe et al., 2017). The present study clearly showed that acupoint specificity is not necessary for the efficacy of TENS treatment, because based on traditional Chinese medicine, the acupoints located in the skin area of the clavicle and scapula were not directly related to gastrointestinal functions. Interestingly, the greatest reduction in VH was seen in the TENS-LL group, whereas no apparent changes were observed in the TENS-RR group, suggesting that the mechanisms underlying TENS-induced reduction of VH may relate to left-right asymmetry. Further studies are needed to determine whether structural and/or functional asymmetry exists at the stimulation sites on the body surface and the other nervous systems involved.

Somatic afferents from the skin at different body sites are involved in the regulation of various autonomic functions, including enhancement and inhibition of gastric motility in cats (Jansson, 1969), rats (Sato et al., 1975; Kamentani et al., 1979), and humans (Camilleri et al., 1984). These observations highlight the importance of cutaneous input

in autonomic control of gastrointestinal functions and suggest a possible topographic relationship between somatic stimulation sites and sympathetic response sites, either excitatory or inhibitory. Thus, the somatosympathetic reflex may play a pivotal role in the mechanisms underlying the TENS-induced reduction of VH in the rats subjected to HIS in the present study.

Although the pathogenesis of IBS remains unclear, most attention is focused on VH related to dysfunction of the autonomic nervous system (Mazur et al., 2007; Manabe et al., 2009; Cheng et al., 2013; Gil et al., 2016). Thus, IBS can be regarded as an imbalance of sympathetic and parasympathetic nervous system functions, and EA or TENS treatment via the somatosympathetic reflex may help restore this balance (Takahashi, 2011). In the present study, we speculate that the coordination and integration of autonomic nervous system function may be involved in the effects of TENS treatment. Further studies are needed to explore the mechanisms of interaction between the sympathetic, parasympathetic, and enteric nervous systems, as well as the balance between sympathetic and parasympathetic functions in the gastrointestinal tract.

Activation of the hypothalamic-pituitary-adrenal (HPA) axis by stress is well documented in humans and rodents. Both physical and psychological stressors can cause the hypothalamus to secrete corticotropin releasing factor (CRF), which acts on the anterior pituitary gland to release adrenocorticotropic hormone (Smith and Vale, 2006). In rodents, adrenocorticotropic hormone can then stimulate the adrenal cortex to secrete corticosterone. A central stress response in HPA axis activity is regulated by circulating glucocorticoid feedback via binding to mineralocorticoid and glucocorticoid receptors at multiple sites in the brain (Harris et al., 2013). Chronic stress reportedly attenuates negative glucocorticoid feedback, disrupting glucocorticoid receptor expression in some brain areas (Mizoguchi et al., 2003) and causing chronic abdominal pain (Johnson et al., 2012). Stereotaxic implantation of corticosterone onto the dorsal margin of the central amygdala leads to chronic VH and colonic dysmotility in rats (Greenwood-Van Meerveld et al., 2001; Venkova et al., 2010). In addition, peripheral injection of CRF induces VH to colorectal distention (La et al., 2008), while intraperitoneal administration of selective CRF² agonists reduces visceromotor response to colorectal distention in rats (Million et al., 2005, 2006), suggesting the participation of a peripheral component of CRF in the development of VH (Larauche et al., 2008). Thus, HPA axis activity reflects a systemic response to stress. It is well established that the HPA axis acts as an important regulatory loop linking the brain and gut, and that stress-induced dysregulation of the HPA axis might play a crucial role in the development of VH. In the present study, TENS at acupoints unrelated to gastrointestinal functions was used to reduce stress-induced VH via the localized somatosympathetic reflex. Whether hormonal actions of the HPA axis are involved in the reduction of VH induced by repeated TENS treatment requires further exploration.

The application of TENS at acupoints has been shown to be more effective at reducing pain than the application of TENS at non-acupoint sites (Chen et al., 1998; Barlas et al., 2006; Cheing and Chan, 2009). TENS applied to the acupoint ST36 restored the decrease in the percentage of normal gastric slow waves (2–4 cycles per minute) induced by cold stress. However, the ameliorating effects on gastric slow waves were not seen when TENS was applied to a non-acupoint located 6 cm away from ST36 (Zhang et al., 2015). Moreover, Furgala et al. (2001) reported that TENS significantly increased the amplitude of electrogastric activity in patients with gastric dysrhythmia when the electrode was placed on the dorsal web between the first and second metacarpal bones (acupoint LI4). However, we found that electrical stimulation of acupoints unrelated to gastrointestinal functions also affected colonic function in rats. This result indicates that the therapeutic effects of TENS may not require specific acupoints. Further clarification of the efficacy of TENS treatment at specific and non-specific acupoints for HIS-induced VH in rats is thus needed.

Overall, the present study revealed that repeated TENS applied to

nonspecific acupoints alleviated stress-induced VH via the somatosympathetic reflex in a rat model of IBS. Further, the right or left side of the upper body where the TENS patches are attached may play an important role in treating gastrointestinal symptoms associated with stress.

Acknowledgments

This study was supported by the Research Foundation of New Chinese Biotechnology Corporation (NCBC-2016-002).

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