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Short communication

Electroacupuncture at Hoku elicits dual effect on autonomic nervous system in anesthetized rats

Jiuan-Miaw Liao [a], Chih-Feng Lin [b], Hua Ting [c], Chi-Chen Chang [a], Yuh-Jeng Lin [d], Tzer-Bin Lin [a,*]

[a] *Department of Physiology, College of Medicine, Chung-Shan Medical and Dental College, No. 110, Chang-Kuo North Rd. 1st Section, Taichung 10018, Taiwan*

[b] *Department of Physical Therapy, College of Medicine, Chung-Shan Medical and Dental College, No. 110, Chang-Kuo North Rd. 1st Section, Taichung 10018, Taiwan*

[c] *Chung-Shan Medical and Dental College Hospital, Taichung, Taiwan*

[d] *Department of Physiology, College of Medicine, National Taiwan University, Taipei, Taiwan*

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Abstract

To address the effect of electroacupuncture (Ea) on the autonomic nerve activity, responses of arteriole blood pressure (BP), intragastric pressure (IGP) and parasympathetic vagal nerve activity (VNA) to Ea were investigated in alpha-chloralose anesthetized rats. The acupoint: Hoku (Li-4) was tested with two different stimulation frequencies (2 and 20 Hz). Decrease in VNA and basal IGP associated with elevation of BP were elicited during Ea at Hoku with stimulation intensity of 20 times of motor threshold. The pattern of response induced by the low frequency Ea (LFEa) was different from that by the high frequency Ea (HFEa), i.e. a tonic effect was elicited by the LFEa, while a phasic one was induced by the HFEa. All the results in this study implicated that: (1) Ea at Hoku may activate the sympathetic and simultaneously inhibit the gastric parasympathetic nerve; (2) Ea at Hoku with different stimulation frequencies may elicit distinct mechanism to induce therapeutic effect; (3) Ea at Hoku may ameliorate the hyperactive stomach in clinical therapy. © 2001 Published by Elsevier Science Ireland Ltd.

Keywords: Electroacupuncture; Hoku; Stomach; Sympathetic nerve; Parasympathetic nerve; Rat

1. Introduction

Acupuncture has been used in China to treat various diseases for more than 2000 years. Many researchers investigating the mechanism underling acupuncture suggested that acupuncture could modulate visceral function through somato-autonomic reflexes (Andersson et al., 1973; Sato and Schmidt, 1987; Sato et al., 1992; Sugiyama et al., 1995).

The effect of electroacupuncture (Ea) on arterial blood pressure (BP) was investigated by several investigators (Ernst and Lee, 1985; Kimura et al., 1995; Kline et al., 1978; Lee and Kim, 1994; Lin and Fu, 2000; Lin et al., 1998; Ohsawa et al., 1995). Lin and Fu suggested pressor effect on BP might be elicited by potentiation of sympathetic tone.

Acupuncture has been successfully employed to suppress stomach hyperactivity in human (Lux et al., 1994; Tougas et al., 1992), neural mechanism underlying the effect of this treatment was investigated by Sato et al. (1993), They suggested segmental sympathetic outflow was activated to induce therapeutic effect.

The major descending influence from the higher center to modulate gastric mobility is through the parasympathetic vagal nerve. In the study stated above, weather the vagal outflow is inhibited or not has not been clearly shown. Therefore, in the present study, the

* Corresponding author. Tel.: +886-4-2473-0022-1652; fax: +886-4-2473-9030.

E-mail address: tblin@mercury.csmc.edu.tw (T.-B. Lin).

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effect of Ea on BP, efferent vagus nerve activity (VNA) and intragastric pressure (IGP) were investigated to differentiate whether sympathetic outflow alone or simultaneously with parasympathetic nervous system was modulated by Ea.

As various visceral functions are generally influenced by stress triggered by various kinds of stimulation, anesthetized animals were employed in this study to eliminate stressful factors.

2. Materials and methods

2.1. General preparation

Twelve adult female SD rats weighting 220–280 g were used throughout this study. All rats in this study had been fasted on food since one day before each experiment, but water was freely accessed. Animals were anesthetized initially with panthrane for surgical preparations, then were maintained under alpha-chloralose anesthesia (50 mg/kg, i.v.). The trachea and the right femoral artery were cannulated for maintaining airway potent and blood pressure recording, respectively.

2.2. Surgical procedures

The abdomen was open by midline section, through a small incision made in the duodenum, a gastric catheter was inserted into the gastric pyloric space and connected to a pressure transducer (P23ID Gould, OH, USA) for IGP recording. In order to record IGP, 2–3 ml saline was infused into stomach to maintain expansion, therefore, stable IGP can be recorded easily, despite it was sightly higher than physiological level. Branches of vagus nerve were dissected from lower esophagus and surrounding tissue, then the lower esophagus was ligated. One small branch of vagus nerve to the stomach was carefully dissected and identified by an increase in IGP contraction ($>10\%$ of control IGP) was elicited by electrical stimulation (20 Hz, 0.5 mA, for 5 s, Fig. 1). After identification, the distal end of this branch was clamped for 5 s using a fine forceps as close as possible to the stomach, and this procedure was repeated at the site 1 mm proximal to the first point alone the nerve fiber (Chien et al., 2000). A paraffin pool was made with skin flaps around the exposed area.

2.3. Stimulation

Acupoint was determined by transposing anatomically from Chinese traditional human acupuncture charts. The acupoint: Hoku, located at the junction of the first and the second metacarpal bones was tested. An interdermal needle (32 gauge, 1/2 inches long, by Trueline Instruments, USA) soldered to a flexible electrical wire, was inserted vertically into the selected acupoint. The second identical needle, as a positive pole, was inserted into the other point approximately 5–10 mm to the first one. Electric current of square wave pulses with pulse duration of 0.05 ms was applied from a stimulator (S88 Grass, RI, USA) through a stimulus isolation unit (SIU5B Grass, RI, USA) and a constant current unit (CCU1A Grass, RI, USA). Two stimulation frequencies: 2 Hz (i.e. low frequency electroacupuncture; LFEa) and 20 Hz (i.e. high frequency electroacupuncture; HFEa) were tested in this study, frequency of 2 Hz was widely employed in manual and electric acupuncture experiments (Araki et al., 1984; Koizumi et al., 1980; Sato and Schmidt, 1987; Sato et al., 1992), while 20 Hz was 10 times of the former to serve as a high frequency stimulation. The stimulation

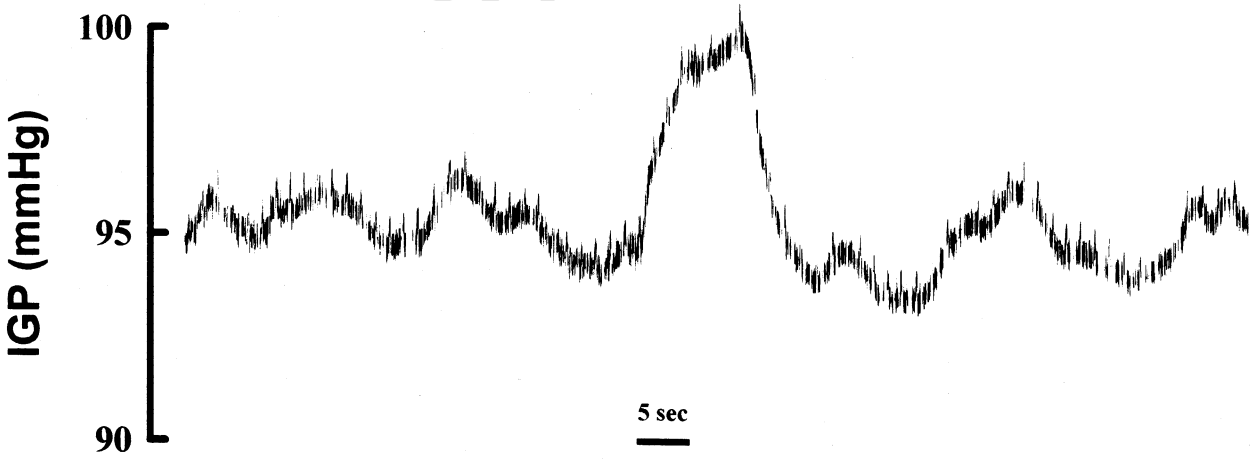

Fig. 1. Identification of vagus nerve innervating stomach. IGP was increased by electric stimulation of vagus nerve. The black bar in the bottom: stimulation for 5 s.

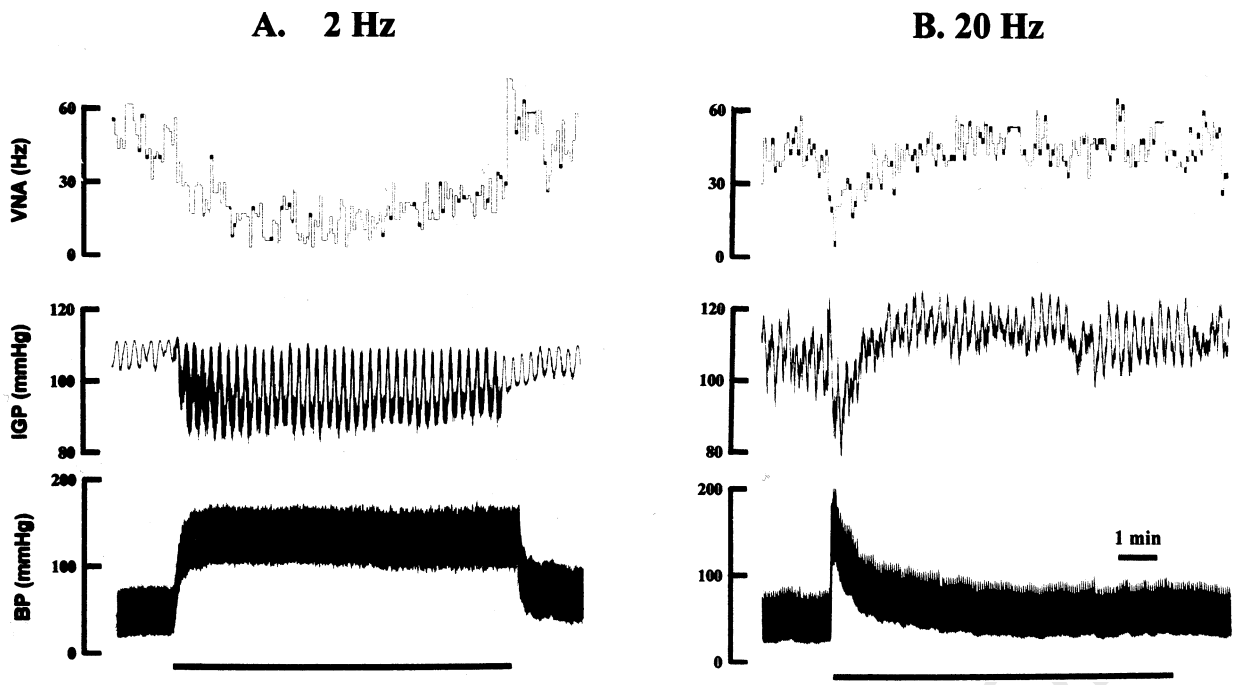

Fig. 2. Effects of Ea on the VNA, IGP and BP. The black bar at the bottom: Ea for 10 min. (A) Following the onset of LFEa ($20 \times T$), a pressor response on BP was elicited slowly and a plateau was reached within 30 s, then maintained until the cessation of LFEa. On the other hand, a decrease in VNA and basal IGP was recorded during stimulation and recovered to control level after the offset of LFEa. (B) Following the onset of HFEa ($20 \times T$), a sharp pressor response on BP was developed rapidly and a peak was reached with 10–15 s, in the meantime, VNA and basal IGP were decreased, then BP, VNA and basal IGP were all gradually recovered to control level in the subsequent 2–3 min.

intensity was set 2, 5 and 20 times of motor threshold ($\times T$; which was minimal intensity to induce muscle contraction), Electric current with higher intensity (40 times of threshold) was not tested for two reasons: (1) it was reported of the same pressor effect as that induced by $20 \times T$ (Lin and Fu, 2000; Lin et al., 1998); (2) it was not suitable for clinical practice in human for it cause intense pain sensation.

2.4. Recording

Multiunit vagal efferent nerve activity was recorded using a pair of thin bipolar stainless wire electrodes. The nerve activities were amplified 100,000-fold and filtered (band pass 30–3000 Hz) by an AC preamplifier (P511AC Grass, RI, USA), and continuously displayed on an oscilloscope (TDS3014 Tectronix, OR, USA). In the meantime, the amplified signals were recorded on a tape recorder (DR-890 Cygnus, PA, USA) and fed into window discriminator (WP5 WPI, FL, USA), the firing frequency of nerve was counted by a spike counter (13-4615-70 Gould, OH, USA) every 5 s and displayed on a recording system (MP30 Biopac LA, USA).

2.5. Statistical analysis

Statistical analysis of the data was performed by means of analysis of variance (ANOVA) following a student's t-test, a probability level of $P < 0.05$ was accepted as significant.

3. Results

3.1. Basal gastic mobility

When the stomach was expanded with saline (2–3 ml), it produced rhythmic fluctuations in IGP with a frequency about 4–10 Hz/min and amplitude of 3–5 mmHg (Fig. 2A). Under the resting condition, fairly stable gastric mobility could be continuous recorded.

3.2. Effect of electoacupuncture

In 5 out of 12 rats, both basal and peak value of IGP fluctuation were decreased by Ea ($20 \times T$), however, in 7 out of 12 rats, only basal IGP was decreased, while the peak IGP showed no significant change to control during Ea. Therefore, basal IGP was employed as an index for Ea effect.

Influence of Ea at Hoku on BP, VNA and basal IGP was shown in Fig. 1A and B, BP was raised while the VNA in parallel with basal IGP was decreased during Ea at Hoku. The patterns of response were different between LFEa and HFEa. Generally, a tonic response was induced by the former, while a phasic one was elicited by the later.

3.3. Effect of low frequency electroacupuncture

BP, VNA and basal IGP in response to LFEa ($20 \times T$) were shown in Fig. 2A. In BP, a pressor response was slowly induced following the onset of stimulation, and a plateau was reached within 30 s ($178 \pm 12.0\%$ of control, $P < 0.01$), then were maintained at this level, until the cessation of stimulation. On the contrast, a decrease of VNA and basal IGP was recorded during LFEa (59.1 ± 5.0 and $72.3 \pm 4.8\%$ of control in VNA and IGP, respectively, $P < 0.01$), and recovered to control level after the offset of stimulation. Effect of LFEa on BP, VNA and basal IGP were summarized in Fig. 3A, with intensity of $20 \times T$, no response was found, however, as long as the stimulation intensity was increased to $5 \times T$, Ea effect described in the above can be elicited. The result induced by Ea with intensity of 5 and $20 \times T$ showed no statistic difference, therefore, were pooled together.

3.4. Effect of high frequency electroacupuncture

BP, VNA and basal IGP in response to HFEa were shown in Fig. 2B. In BP, a sharp pressor response was developed rapidly following the onset of stimulation, and a peak was reached within 10–15 s ($209 \pm 16.6\%$ of control, $P < 0.01$), VNA and basal IGP was decreased at the early period of HFEa (52.8 ± 4.2 and $55.0 \pm 5.4\%$ in VNA and IGP, respectively, $P < 0.01$), then BP, VNA and basal IGP were all gradually recovered to the control level in the subsequent 2–3 min. Effects of HFEa on BP, VNA and basal IGP were summarized

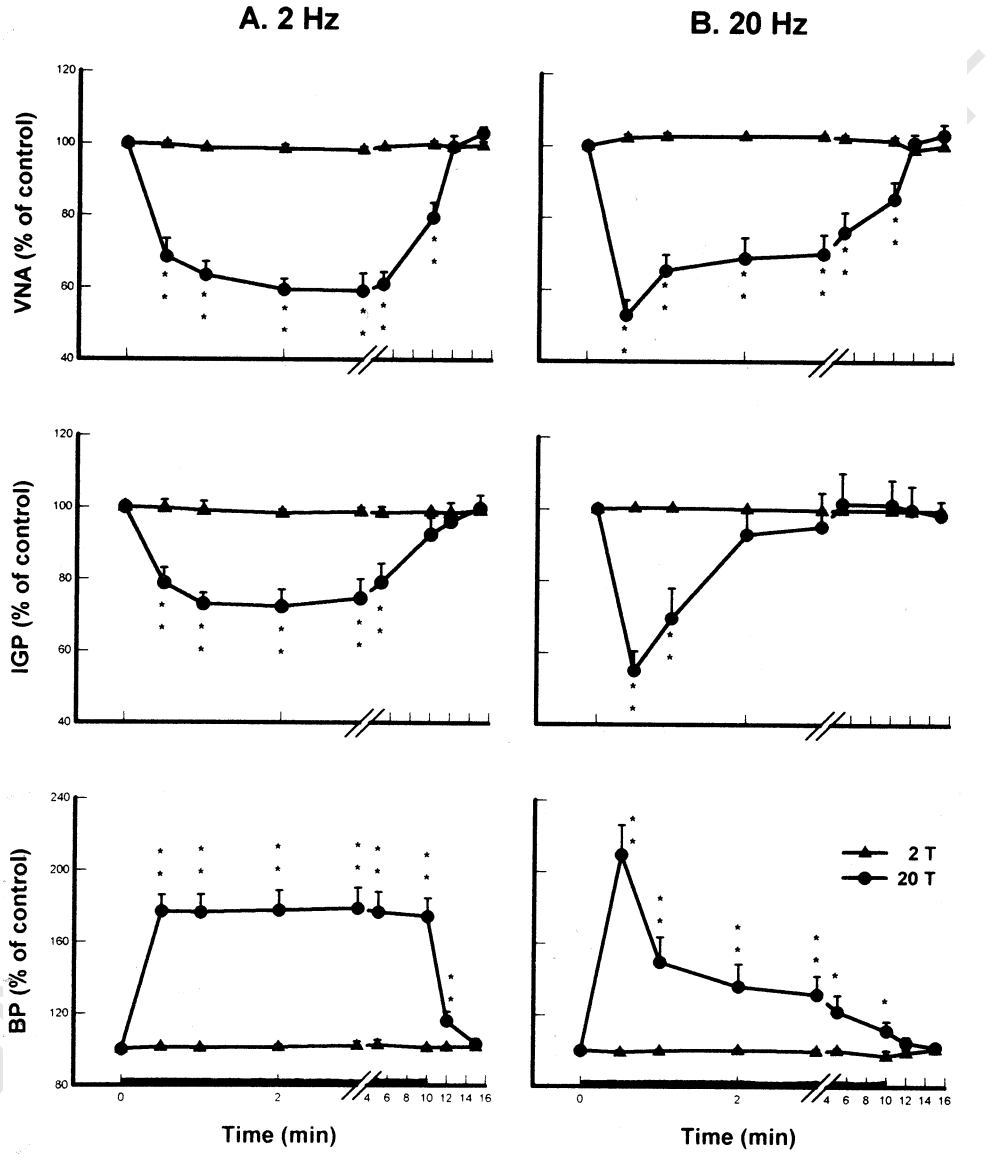

Fig. 3. Effects of Ea on VNA, basal IGP and BP. Note: a tonic effect was elicited by LFEa (A), while a phasic one was did by HFEa (B). The black bar in abscissa: Ea for 10 min. (▲, $2 \times T$; ●, $20 \times T$; $^{**}P < 0.01$, $n = 8$).

in Fig. 3B. With intensity of $2 \times T$ HFEa caused no effect, either. HFEa with intensity of 5 and $20 \times T$ caused a phasic pressor effect on BP associated with phasic decrease in VNA and basal IGP. The result caused by HFEa with intensity of 5 and $20 \times T$ showed no significant difference, therefore were once again pooled together.

3.5. Bilateral vogotomy

Both the inhibitory effect elicited by LFEa and HFEa on VNA and IGP was blocked by bilateral vogotomy at the cervical level, while that on BP was not affected.

4. Discussion

In the present study, we demonstrated that a pressor response of BP associated with inhibition on the VNA and basal IGP was elicited by Ea at Hoku. The effect elicited by LFEa and HFEa was different, i.e. a tonic effect was elicited by the former, while a phasic one was induced by the later.

4.1. Effect on autonomic nervous system

Many researchers exploring the physiological mechanism underlying acupuncture suggested that acupuncture might modulate the nerve activity influencing visceral function via somato-autonomic reflexes (Andersson et al., 1973; Sato and Schmidt, 1987; Sato et al., 1992; Sugiyama et al., 1995). Lin and Fu recorded the sympathetic renal and adrenal nerve as well as arteriole BP in anesthetized rat, and reported that Ea at Hoku may induce pressor response by potentiation of sympathetic tone (Lin and Fu, 2000; Lin et al., 1998). In this study, pressor effect on BP was also induced by Ea, this was thought to be a result of excitation in the sympathetic outflow. On the other hand, when the BP was increased, there was a synchronized decrease in parasympathetic VNA and basal IGP elicited. Since gastric vagal branches have a lot of sympathetic contamination, bilateral vagotomy at cervical level was performed to rule out the sympathetic effect on IGP. In these experiments, pressor effect on BP can be elicited by Ea as that in intact rats, while the inhibitory effect on IGP was abolished, i.e. vagal efferents were the main route involved in the inhibitory effect elicited by Ea. These results implicated not only sympathetic tone was potentiated by Ea, but also the parasympathetic outflow, at least the parasympathetic vagal outflow to stomach was inhibited simultaneously, i.e. a dual modulatory effect was induced.

4.2. Possible neural pathway involved

In our opinion, this dual modulatory effect may be resulted from a somato-autonomic reflex mediating by the central nervous system, i.e. electric stimulation activated the somatic afferents, after transmitting the nerve impulses into the higher center, two agonistic effect were elicited: the first was the sympathetic outflow was generally excited and elicited a pressor response on BP. The second was the parasympathetic nervous system was inhibited at the same time, therefore, a parallel inhibitory response in VNA and basal IGP was induced.

4.3. Stimulation intensity

The stimulation intensity employed in this study to induce such a dual modulatory effect was higher than $5 \times T$ (i.e. 5 and $20 \times T$), Ea with intensity of $2 \times T$ which activates group I afferent fiber was of no effect. Electric impulse with intensity of 5 and $20 \times T$ was sufficient to recruit group II and III afferents (Lin and Fu, 1998), This result was correlated with several reports suggested group II and III was the major sensory input to elicited Ea effect (Lin and Fu, 2000; Lin et al., 1998; Sato and Schmidt, 1987; Sato et al., 1992).

4.4. Low and high frequency electroacupuncture

Both LFEa and HFEa employed in this study were able to elicit dual modulatory effect, however, a tonic effect was induced by LFEa, while a phasic one was did by HFEa. Lin and Fu using combinations of LFEa and HFEa either simultaneously or with a time lag to explore the possible mechanism involved in these two types of stimulation and suggested the central mechanism was independent each other in LFEa and HFEa (2000). Chen et al. (1996) suggested that excepted sharing the same central mechanism with LFEa to activate mu and delta opioid receptors, HFEa was able to activate kappa receptor which was not excited by LFEa, which means that Ea with various frequency may elicit different response at the identical stimulation site (1996). This may be the reason why different patterns of inhibitory effect were elicited by LFEa and HFEa in this study. However, the precise neural pathway and the centers involving in this reflex need more experiments to be clarified.

4.5. Amplitude of peristaltic contraction

In the present study, the basal and mean IGP was decreased by Ea at Hoku, however, the amplitude of peristaltic contraction was increased during Ea. The peak pressure of each contraction was at about the same level as that in control. To our knowledge, the

mechanism involved in such phenomenon was unclear, however, may be resulted from the 'receptive relaxation' mediated by the myenteric plexus in gastrointestinal tract which antagonistic contraction was carried out successively along hallow organs. Since the basal tone was significantly decreased by Ea, antagonistic contraction was compensatorily increased to maintain the peak pressure as the control level. However, the precise mechanism needs further investigation.

5. Conclusion

In conclusion, our results indicate that: (1) Ea at Hoku may activate sympathetic and simultaneously inhibit parasympathetic nervous system; (2) Ea at Hoku with different stimulation frequency may elicited a distinct mechanisms to induce such dual modulatory effect; and (3) Ea at Hoku may ameliorate hyperactive stomach in clinical therapy.

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