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Pressor Effects on Blood Pressure Induced by Isovolumic Bladder Distension and Electro-Acupuncture Stimulations in Anesthetized Rats

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Abstract

The pressor effects on blood pressure (BP) elicited by electro-acupuncture (Ea) stimulations and vesico-vascular reflex (VVR) were investigated in anesthetized rats. Twenty adult female Sprague-Dawley rats were used throughout this study. Two acupoints, the Hoku (Li-4) and the Tsusanli (St-36), were tested by a low frequency Ea (LFEa, 2 Hz) and a high frequency Ea (HFEa, 20 Hz) with intensities 20 times that of the motor threshold. Ea at Tsusanli elicited no pressor effects (98.15 ± 4.10% and 101.43 ± 3.96% of pre-stimulation control in LFEa and HFEa, respectively) whereas pressor effects could be induced by Ea stimulations at Hoku (126.3 ± 3.3% and 136.3 ± 3.8% of pre-stimulation control in LFEa and HFEa, respectively, $P < 0.01$, n = 10). In addition, the patterns of pressor effects elicited by LFEa and HFEa at Hoku were different, *i.e.*, LFEa at Hoku elicited a tonic pressor effect, while HFEa elicited a phasic one. The VVR induced by bladder isovolumic saline distension also elicited a pressor effect on BP (119.2 ± 2.2%, $P < 0.01$, n = 10) in the same preparations during bladder contractions. The VVR did not modify the Ea-induced pressor responses, and vice versa, when both of them were superimposed. All the results suggested that the pressor effects elicited by the VVR and the Ea stimulation were additive responses. In addition, for future clinical application, our findings may imply that patients should be carefully monitored for signs and symptoms of autonomic dysfunction during clinical acupuncture treatments.

Key Words: bladder, electro-acupuncture, Li-4, St-36, vesico-vascular reflex, blood pressure, rats

Introduction

Urine retention resulting from a spastic bladder is one of the major problems in suprasacral spinal cord injury (SCI) patients. Bladder-distension of the urinary bladder has been shown to cause a reflex

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increase in sympathetic nerve outflow and results in a pressor effect on blood pressure (BP) and is known as the vesico-vascular reflex (VVR) (2, 16, 18). A risk of VVR in SCI patients with high neurologic level is the possible occurrence of autonomic dysreflexia that somatic or visceral stimuli trigger excessive sympathetic discharges resulting in hypertension, sweating and headaches. Acute hypertension can be potentially dangerous and has grave consequences including stroke and seizure and, therefore, must be monitored closely and treated as a medical emergency. VVR can be elicited in anesthetized normal (3, 5) as well as animals with spinal cord injury (7); the former has recently been reported as a useful model for studying autonomic dysreflexia (4).

In China, acupuncture has been used to treat various diseases for more than two thousand years. Nowadays, it has been recognized by the National Institutes of Health (NIH) of the U.S.A. as potentially useful for a variety of chronic pain conditions or for patients for whom conventional treatments have proven ineffective. Because there are almost no adverse effects associated with acupuncture stimulations in the general population, it is a reasonable choice for some patients with chronic pain.

Researchers investigating the mechanism underlying acupuncture have suggested that acupuncture stimulations might modulate nerve activities influencing visceral functions *via* somato-sympathetic reflexes (1, 6, 8, 17, 19). Studies on the effects of acupuncture stimulations on the cardiovascular system have demonstrated that acupuncture stimulation elicited pressor effects on human subjects (9, 13, 20-22) and anesthetized rats (12, 14, 23). Lin and Fu (14, 15) investigated the mechanism underlying electro-acupuncture (Ea) and suggested that pressor effects were elicited by an Ea-induced potentiation in sympathetic tone. In addition, Sato *et al*. (19) and Liao *et al*. (11) investigated the therapeutic effects induced by Ea on hyperactive stomachs, and also suggested that segmental sympathetic outflow was activated by Ea to induce therapeutic effects.

Because both VVR and Ea stimulations might excite the sympathetic nervous system to induce pressor effects, the focus of the present study is on whether Ea may facilitate the risk of autonomic dysfunction in an animal model with an over-distended bladder. In addition, the interaction between these two reflexes was also investigated.

Materials and Methods

General Operations

Twenty adult female Sprague-Dawley rats, weighing 180-360 g, were used throughout this study. The animals were anesthetized with urethane (1.2 g/kg, i.p.). The left femoral artery was cannulated for BP recording, and the right femoral vein and trachea were also done for anesthetic administration and for maintaining of airway potent, respectively. The systemic BP was continuously recorded on a computer system (Biopac MP30, Biopac, Santa Barbara, CA, USA) through a transducer (Statham P23 ID, Oxnard, CA, USA) with an arterial catheter. The rectal temperature was maintained at around 37°C using an infrared lamp.

Electro-Acupuncture Stimulations

Acupoints were determined by anatomical transposition from traditional Chinese human acupuncture charts. Two acupoints: the Tsusanli (St-36), located at the lateral upper tibia, and the Hoku (Li-4), located at the junction of the first and the second metacarpal bones were tested. An inter-dermal needle (32 gauge, 1/2 inches long, by Trueline Instruments Inc.,) soldered to a flexible electrical wire was inserted vertically into the selected acupoints. A second identical needle, as a positive pole, was inserted into the other point approximately 5 to 10 mm from the first one. Ea stimulations were applied from a stimulator (Grass S88, Cleveland, OH, USA) through an isolation unit (Grass SIU5B) and a constant current unit (Grass CCU1A) to provide electric currents (square wave pulses with durations of 0.05 ms). Two stimulation frequencies, 2 Hz and 20 Hz, were tested in this experiment; a frequency of 2 Hz (low frequency Ea; LFEa) was widely employed in manual and electric acupuncture studies, and a frequency of 20 Hz (high frequency Ea; HFEa), 10 times that of the former, served as a high frequency stimulation. The stimulation intensity was 20 times the threshold (the minimal stimulation intensity to induce muscle twitch) for this intensity can recruit afferent efficiently to induce maximal pressor effect (14). In this study the total stimulation time in this study was set for 10 min because the effects of Ea on BP became stable within 5 min.

Cystrometry Investigations

A polyethylene catheter (PE-50, Portex, Hythe, Kent, UK) was inserted into the bladder through the urethra and residual urine was removed. The urethra was ligated to the catheter near the external urethral meatus. The intravesicular pressure (IVP) was monitored *via* the urethral catheter that was connected to a pressure transducer (Statham P23) and a saline-infusion pump through a three-way stop-cock. The bladder was filled with physiological saline (0.06 ml/min) until a volume that induced spontaneous bladder

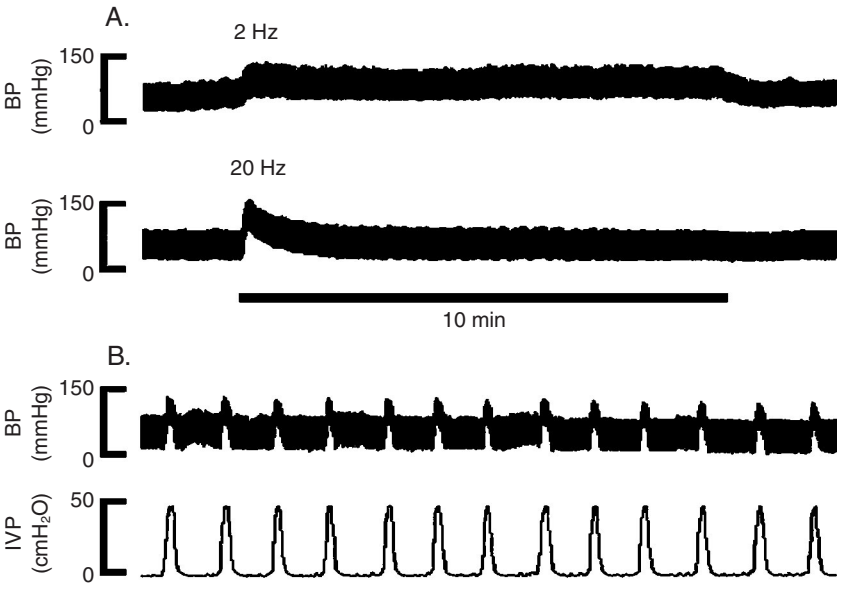

Fig. 1. (A) Pressor effects on blood pressure (BP) elicited by electro-acupuncture stimulations (indicated by the black bar at the bottom). The upper trace: following the onset of a low frequency electro-acupuncture (2 Hz), a pressor response was slowly induced and maintained at this level until cessation of the stimulation. The lower trace: a sharp pressor response was induced following the onset of a high frequency electro-acupuncture (20 Hz) and gradually returned to the pre-stimulation control value at about 3 min after the stimulation onset. (B) Pressor effects on blood pressure elicited by bladder isovolumic contractions (IVCs). Rhythmic ICVs induced by bladder saline distension elicited pressor effects on blood pressure in parallel with intravesicular pressure (IVP) increase.

contractions (referred as the "volume threshold"). Then the bladder was drained free and the animal was rested for an equilibrium period (10 min). Saline with a volume of 1.5 times volume threshold was instilled into the urinary bladder through the urethral catheter to elicit rhythmic isovolumic bladder contractions (IVCs) (10).

Statistical Analysis

Statistical differences between groups were determined using a two-way analysis of variance (ANOVA) followed by Student's *t*-test and $P < 0.05$ was accepted as a minimal level of significance.

Results

Effects of Electro-Acupuncture at Hoku

LFEa and the HFEa at Hoku both elicited pressor effects on BP during the stimulation periods. However, the patterns of the pressor responses induced by these two Ea stimulations were distinctly different from one another (Fig. 1A). BP in response to the LFEa is shown in the upper trace of Fig. 1A. Following the onset of the LFEa, a pressor response was slowly induced and a maximum effect was reached within 30 sec (126.3 ± 3.3% of pre-stimulation control, n = 10); BP was maintained at this level until cessation of the stimulation. The pressor effects elicited by the LFEa are summarized in Fig. 3A (reversed triangle). On the other hand, the BP in response to the HFEa is shown in the lower trace of Fig. 1A. A sharp pressor response was induced following the onset of stimulation. The peak effect was reached within 10-15 sec (136.3 ± 3.8% of pre-stimulation control, n = 10). Then BP gradually returned to the pre-stimulation control level in the subsequent 2-3 min. The pressor effects elicited by HFEa are summarized in Fig. 3B (reversed triangle). All the pressor responses were abolished by cutting the radial nerve trunk central to the ipsilateral stimulated Hoku points (n = 3).

Effects of Ea at Tsusanli

At Tsusanli, neither the LFEa nor the HFEa induced pressor effects on BP (98.15 ± 4.10% and 101.43 ± 3.96% of pre-stimulation control in LFEa and HFEa, respectively).

Effects of Bladder Distension

As shown in Fig. 1B, saline distension induced rhythmic isovolumic contractions (IVCs; 1.8 ± 0.7 contractions/min) in the urinary bladder. In addition,

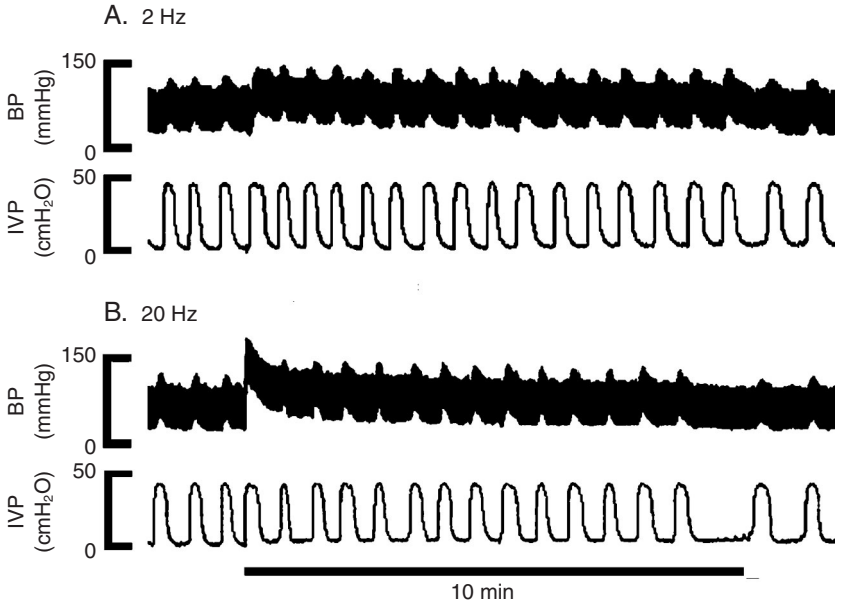

Fig. 2. Pressor effects on blood pressure (BP) elicited by bladder isovolumic contractions (IVCs) superimposed on electro-acupuncture stimulations (indicated by the black bar at the bottom). In addition to the pressor effects elicited by the low frequency (A. 2 Hz) and the high frequency (B. 20 Hz) electo-acupuncture stimulation, each IVC induced a further elevation in blood pressure during the stimulation period.

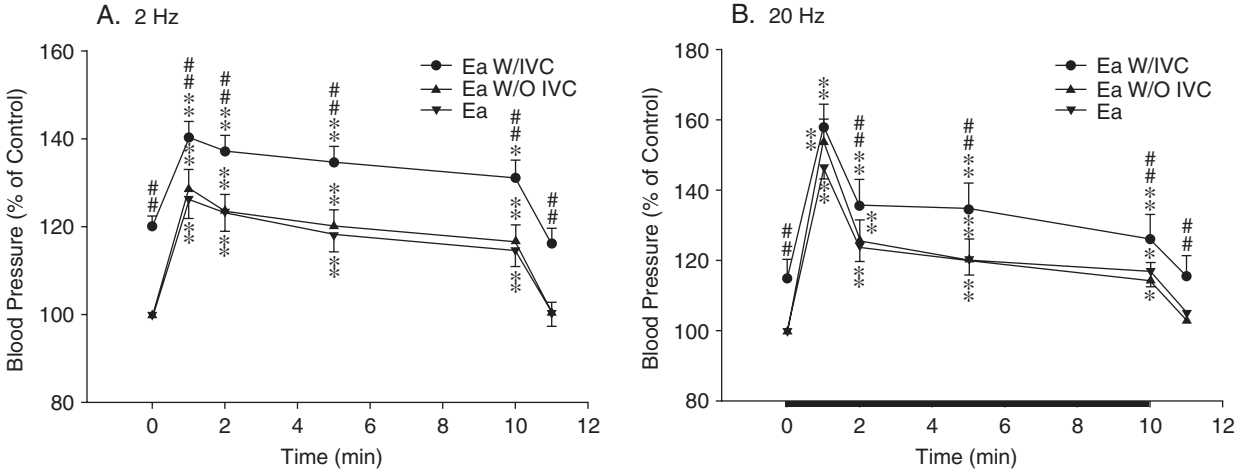

Fig. 3. The effects of superimposed IVCs on electro-acupuncture-induced pressor response. The pressor effects on blood pressure (BP) elicited by IVCs superimposed on electro-acupuncture stimulations (Ea W/ IVC; circle), bladder distention without IVC during electro-acupuncture stimulation (Ea W/O IVC; triangle) and electro-acupuncture stimulation alone (Ea; reversed triangle) summarized from 10 rats in a low frequency (A. 2 Hz) and a high frequency (B. 20 Hz) electro-acupuncture stimulations. The pressor effects elicited by the electro-acupuncture stimulations with IVCs were significantly higher than the electro-acupuncture stimulations alone and the electro-acupuncture stimulations under bladder distension but without IVC (*$P < 0.05$, **$P < 0.01$ significant different from pre-stimulation control; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, significant different from electro-acupuncture stimulation alone, n = 10). However, no statistical significance was shown between the latter two conditions.

each IVC elicited a pressor effect on BP (119.2 ± 2.2% of control, n = 10) in parallel with increasing intravesical pressure.

Combinations of Eas and Bladder Distension

As shown in Fig. 2, we tested the pressor effects induced by a combination of the Ea stimulations and bladder distension. In addition to the pressor effects elicited by the Ea stimulations, each IVC elicited a further elevation of BP during Ea stimulation (Fig. 2A, 2 Hz and 2B 20 Hz), *i.e.*, IVCs elicited additive pressor effects on Ea stimulation. On the other hand, during the latencies between the two IVCs, the pressor

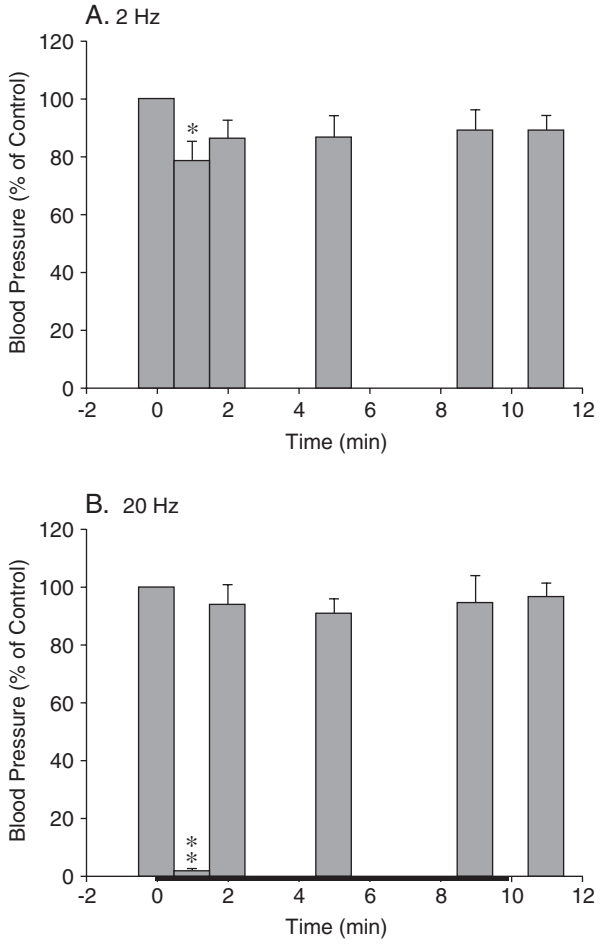

Fig. 4. Pressor effects on blood pressure (BP) induced by the isovolumic contractions (IVCs) in pre-stimulation control stage and during electro-acupuncture stimulations (indicated by the black bar at the bottom). The pressor effects elicited by IVCs during the simulation period of the low frequency (A. 2 Hz) and the high frequency (B. 20 Hz) electro-acupuncture stimulations showed no statistical difference to that of pre-stimulation control excepting at the onset stage (*$P < 0.05$, **$P < 0.01$ significant different from pre-stimulation control, n = 10).

effects elicited by Eas combined with bladder distension were similar to that done by Eas alone. As summarized in Fig. 3, both in the LFEa (A. 2Hz) and HFEa (B. 20 Hz), pressor effects elicited by IVCs superimposed on Ea stimulations were significantly higher than Ea stimulations alone (119.2 ± 2.2%, $P < 0.01$, n = 10). However, no statistical significance was shown between Ea stimulations alone and Eas under bladder distension without IVCs.

The pressor effects induced by IVCs in the pre-stimulation control stage and during Ea stimulations under a combination of Ea and bladder distension are summarized in Fig. 3. Excepting at the onset stage (the first 30 sec after Ea started), the amplitude of IVC-induced pressor effects showed no statistical differences to that of pre-stimulation control (Fig. 4).

Discussion

The present study demonstrates that Ea stimulations at the Hoku elevated BP while stimulations with identical parameters at Tsusanli caused no effects. Ea with frequencies of 2 and 20 Hz elicited different patterns of pressor effects, *i.e.*, LFEa induced a tonic pressor effect, while HFEa induced a phasic one. On the other hand, IVCs induced by volume distension could elicit additive pressor effects on the same preparations during Ea stimulations.

In the present study, the pressor effects elicited by IVCs superimposed on Ea stimulations were higher than that done by Eas alone. No statistical difference was shown between Ea alone and Ea under bladder distension without IVC. This implies that the pressor effects elicited by Ea stimulations were not affected by IVCs. On the contrary, during the maintained stages of Ea stimulations (from the first minute to the cessation of Ea), the amplitude of the pressor effects elicited by IVCs showed no difference to that in pre-stimulation control stage indicating that the amplitude of pressor effects elicited by IVCs were independent of Ea stimulations. According to the results stated above, at least during the maintained stage of Ea stimulations, because even if the central pathways mediating the reflexes associated with Ea- and IVC-induced pressor effects were not entirely separate, an additive effect could still occur. Therefore, the detailed mechanism needs to be further investigated.

In the present study, the pressor effects elicited by Ea stimulations were higher than that by the IVC (*i.e.*, 126.3 ± 3.3% in LFEa, 136 ± 3.8% in HFEa and 119.2 ± 2.2% in IVC). This may imply that the neurotransmitter triggered by IVC was far less than that by Ea stimulations. In our conjecture, at the onset stage, Ea stimulations induced the release of large amounts of catecholamine to cause vasoconstriction in systemic arteries resulting in an increase in BP. During this period, the neurotransmitter that can be triggered by the IVC was less; therefore, no significant additive effects were induced. On the other hand, after this stage, BP was maintained at a constant level (LFEa) or even gradually recovered to the control value (HFEa), *i.e.*, Ea stimulations no longer caused extra contractions of vascular smooth muscles for the neurotransmitter being released was reduced. During this stage, despite only a small amount of catecholamine triggered by the IVC, such a release can cause significant pressor effects on BP. This can also explain why Ea stimulations masked the pressor effects elicited by the IVC at the early stage while the Ea-induced pressor response was not affected by the

IVCs. Furthermore, this may also be the reason why, as shown at the onset stage in Fig. 2, an HFEa almost completely masked, while a LFEa attenuated, the IVC-induced pressor effects at the onset stage.

Safety, practicality and versatility make acupuncture a useful tool for specialists treating complicated pain presentations in the general population. However, autonomic dysfunction in association with the acupuncture has been reported in the recent literature (1). Clinically, an acute elevation in blood pressure of 20 to 40 mmHg above the baseline value may be considered a sign of autonomic dysfunction (1). In this study, we find that the pressor effects elicited by IVCs may superimpose on Ea stimulations and higher than that done by Eas alone. The results demonstrate that in the bladder-distended preparations, at least during the maintained phase of Ea stimulations, the occurrence of autonomic dysfunctions was considered a potential risk. Therefore, for future clinical application, our findings may imply that patients should be carefully monitored for signs and symptoms of autonomic dysfunction during clinical acupuncture treatments. However, the animal model used in the present study is one involving anesthesia, and that is quite different from patients with clear consciousness in clinical practice; hence, the risk of electro-acupunture on SCI patients should be further investigated.

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