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Pressor effects on blood pressure induced by isovolumic bladder distension and electroacupuncture stimulations in anesthetized rats

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Running title: Distension- and stimulation-induced pressor effects

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Key words: urinary bladder, acupuncture, Li-4, St-36, Blood pressure, Rat

Abstract

The pressor effects on blood pressure (BP) elicited by the electroacupuncture (Ea) stimulations and vesico-vascular reflex (VVR) were investigated in anesthetized rats. Two acupoints, the Hoku (Li-4, at the junction of the first and the second metacarpal bones) and the Tsusanli (St-36, at the lateral upper tibia bone) were tested by a low frequency Ea (LFEa, 2 Hz) and a high frequency Ea (HFEa, 20 Hz) with intensities 20 times the motor threshold. Ea at Tsusanli elicited no pressor effects (98.15±4.10% and 101.43±3.96% of prestimulation control in LFEa and HFEa, respectively, $p<0.01$, N=10), whereas, pressor effects can be induced by Ea stimulations at Hoku (126.3±3.3% and 1136.3±3.8% of prestimulation control in LFEa and HFEa, respectively, $p<0.01$, N=10). In addition, the patterns of pressor effects elicited by a LFEa and a HFEa at Hoku were different, i.e., a LFEa at Hoku elicited a tonic pressor effect, while a HFEa did 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 the pressor effects elicited by the VVR and the Ea stimulation were additive responses. In addition, patients should be carefully monitored for signs and symptoms of autonomic dysfunction during clinical acupuncture treatments.

Key words: urinary bladder, acupuncture, Li-4, St-36, Blood pressure, Rat

1. Introduction

Urine retention resulted 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 increase in sympathetic tone and result in a pressor effect on blood pressure (BP), which is known as the vesico-vascular reflex (VVR; Marry, 1989; Cervero, 1993; Lin et al., 1998). A risk of the 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 headache. Acute hypertension can be potentially dangerous and have grave consequences including stroke and seizure, and therefore, must be monitored closely and treated as a medical emergency.

In China, the acupuncture has been used to treat various diseases for more than two thousand years. Nowadays, it has been recognized by National Institutes of Health (NIH) of U.S.A. as potentially useful for a variety of chronic pain conditions or for patients in whom conventional treatments are 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 suggested acupuncture stimulations might modulate nerve activities influencing visceral functions via somato-sympathetic reflexes (Averill et al., 2000; Kimura et al., 1995; Langevin et al., 2002; Loaiza, 2002; Sato et al., 1993). Studies on the effects of acupuncture stimulations on cardiovascular system have demonstrated acupuncture stimulation elicited pressor effects on human subjects (Lee et al., 2003; Stener-Victorin et al., 2003a;b ; Sugiyama et al., 1995) and anesthetized rats (Lin et al., 1998; Ting et al., 2002). Lin and Fu (1998; 2000) investigated the mechanism underlies the electroacupuncture (Ea) suggested pressor effects were elicited by an Ea-induced potentiation in sympathetic tone. In addition, Sato et al. (1993) and Liao et al. (2001) investigated the therapeutic effects induced by Ea on hyperactive stomach also suggested segmental sympathetic outflow was activated by the Ea to induce therapeutic effects.

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

2. Material and Methods

2.1. General operations

Twelve adult female Sprague-Dawley rats, weighing 180-360g, were used throughout this study. Animals were anesthetized with urethane (1.2 g/kg, *i.p.*). Urethane was chosen because it lacks ganglionic blocking properties, therefore, allows the extrinsic neural innervations to/from the visceral organs to be maintained. The left femoral artery was cannulated for BP recording, the right femoral vein and trachea were also done for anesthetic administration and maintaining of airway potent, respectively. The systemic BP was continuously recorded on a computer system (Biopac MP30) through a transducer (Statham P23) with an arterial catheter. The opened abdominal cavity was covered with warm paraffin oil to avoid drying. The rectal temperature was maintained at around 37°C using an infrared lamp.

2.2. Electroacupuncture Stimulations

Acupoints were determined by transposing anatomically from Chinese traditional 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 interdermal needle (32 gauge, 1/2 inches long, by Trueline Instruments) soldered to a flexible electrical wire, was inserted vertically into the selected acupoints. The second identical needle, as a positive pole, was inserted into the other point approximately 5 to 10 mm to the first one. Electric currents of square wave pulses with durations of 0.05 ms were applied from a stimulator (Grass S88) through a stimulus isolation unit (Grass SIU5B) and a constant current unit (Grass CCU1A). 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) was 10 times of the former to serve as a high frequency stimulation. The stimulation intensity was 20 times the threshold (the minimal stimulation intensity to induce muscle twitch). The total stimulation time in this study was set for 10 min because the effects of Ea on BP became stable within 5 min in this study.

2.3. Cystrometry investigations

A transurethral bladder catheter connected to a pressure transducer was used to record the intravesicular pressure (IVP) isovolumically with the urethral outlet ligated (Lin 2003; 2004). Rhythmic isovolumic bladder contractions were induced by infusion with saline of 1.5 times volume threshold (the minimal volume that induced bladder voiding contractions) into the urinary bladder.

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

3. Results

3.1. Effects of electroacupucture 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 distinct from each other (figure 1A). BP in response to the LFEa was shown in the upper trace of figure 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), then BP was maintained at this level until the cessation of stimulation. The pressor effects elicited by the LFEa were summarized in figure 3A (reversed triangle). On the other hand, the BP in response to the HFEa was shown in the lower trace of figure 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 was gradually returned to the pre-stimulation control level in the subsequent 2-3 min. The pressor effects elicited by HFEa were summarized in figure 3B (reversed triangle).

3.2. Effects of Ea at Tsusani

At Tsusanli, neither the LFEa nor the HFEa induced pressor effects on BP (98.15±4.10% and 101.43±3.96% of prestimulation control in LFEa and HFEa, respectively, $p<0.01$, N=10).

3.3. Effects of bladder distension

As shown in figure 1B, saline distension induced rhythmic isovolumic contractions (IVCs; 1.8±0.7 contractions/min, N=10) in the urinary bladder. In addition, each IVC elicited a pressor effect on BP (119.2±2.2% of control, N=10) in parallel with intravesicular pressure increase.

3.4. Combinations of the Eas and bladder distension

As shown in figure 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 in BP during Ea stimulation (figure 2A 2Hz and 2B 20 Hz), i.e., IVCs elicited an additive pressor effects on Ea stimulation. On the other hand, during the latencies between each two IVCs, the pressor effects elicited by Eas combined with bladder distension were similar as that done by Eas alone. As summarized in figure 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 (## 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 pre-stimulation control stage and during Ea stimulations under a combination of Ea and bladder distension were summarized in figure 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.

DISCUSSION

The present study demonstrated 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., a LFEa induced a tonic pressor effect, while a HFEa did a phasic one. On the other hand, IVCs induced by volume distension can 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 was higher than that did 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 the amplitude of pressor effects elicited by IVCs were independent to Ea stimulations. According to the results stated above, we suggested, at least during the maintained stage of Ea stimulations, the central mechanisms involved in Ea- and IVC-induced pressor effects seem to be distinct.

The reason why Ea stimulations attenuated the IVC-induced pressor effects at the early stage of stimulation is still unclear. Lin and Fu (2000) investigated the possible efferent pathways involved in Ea-induced pressor effect using adrenlectomized rats. They suggested a general excitation of sympathetic vasomotor tone but not the neurohumoral release of catecholamine from the adrenal medulla underlies the Ea-induced pressor effects. In the present study, although the central mechanisms underlie the IVC- and the Ea-induced pressor effects might be different, the final pathway(s) seems to be common (Ting et al., 2002; Lin 2003; Lin et al., 2003) i.e., the excitation of sympathetic ending to induce vasoconstriction. 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 the neurotransmitter triggered by IVC was far less than that by Ea stimulations. In our conjecture, at the onset stage, Ea stimulations induced large amount of catecholamine release to cause the vasoconstriction in systemic arteries, that resulting an increase in BP. During this period, the neurotransmitter 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 in a constant level (LFEa) or even gradually recovered to the control value (HFEa), i.e., Ea stimulations caused no longer extra contractions of vascular smooth muscles for the neurotransmitter released was reduced. During this stage, despite only a small amount of catecholamine is triggered by the IVC, such release can cause a significant pressor effect 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 maybe also the reason why, as shown at the onset stage in figure 2, a HFEa almost completely masked, while a LFEa attenuated the IVC-induced pressor effects at the onset stage. However, the detailed mechanisms involved need further investigations.

Safety, practically and versatility make the acupuncture a useful tool for specialists treating complicated pain presentations in the general population. However, the autonomic dysfunction in association with the acupuncture has been reported in the recent literature (Averill et al., 2000). The results in the present study demonstrated that in the bladder-distended preparations, at least during the maintained phase of Ea stimulations, the occurrence of autonomic dysfunctions was considered to be a potential risk. Therefore patients should be carefully monitored for signs and symptoms of autonomic dysfunctions during clinical acupuncture treatments.

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Figure legend

Figure 1. (A) the pressor effect on blood pressure (BP) elicited by the electroacupuncture stimulations (indicated by the black bar at the bottom). The upper trace: following the onset of a low frequency electroacupuncute (2 Hz), a pressor response was slowly induced and maintained at this level until the cessation of stimulation. The lower trace: a sharp pressor response was induced following the onset of a high frequency electroacupuncture (20 Hz) and gradually returned to the pre-stimulation control value at about 3 min after stimulation onset. (B) The 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 intra-vesicular pressure (IVP) increase.

Figure 2. The pressor effects on blood pressure (BP) elicited by bladder isovolumic contractions (IVCs) superimposed on electroacupuncture 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) electoacupuncture stimulation, each IVC induced a further elevation in blood pressure during the stimulation period.

Figure 3. The effects of superimposed IVCs on electroacupuncture-induced pressor response. The pressor effects on blood pressure (BP) elicited by IVCs superimposed on electroacupuncture stimulations (Ea W/ IVC; circle), bladder distention without IVC during electroacupuncture stimulation (Ea W/O IVC; triangle) and electoacupuncture stimulation alone (Ea; reversed triangle) summarized from 10 rats in a low frequency (A. 2 Hz) and a high frequency (B. 20 Hz) electroacupuncture stimulations. The pressor effects elicited by the electroacupuncture stimulations with IVCs were significantly higher than the electroacupuncture stimulations alone and the electroacupuncture 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 electroacupuncture stimulation alone, N=10). However, no statistical significance was shown between the latter two conditions.

Figure 4. The pressor effects on blood pressure (BP) induced by the isovolumic contractions (IVCs) in pre-stimulation control stage and during electroacupuncture 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) electroacupuncture stimulations showed no statistical difference to that of pre-stimulation control excepting at the onset stage (** $p<0.01$; * $p<0.05$ significant different from pre-stimulation control, N=10).

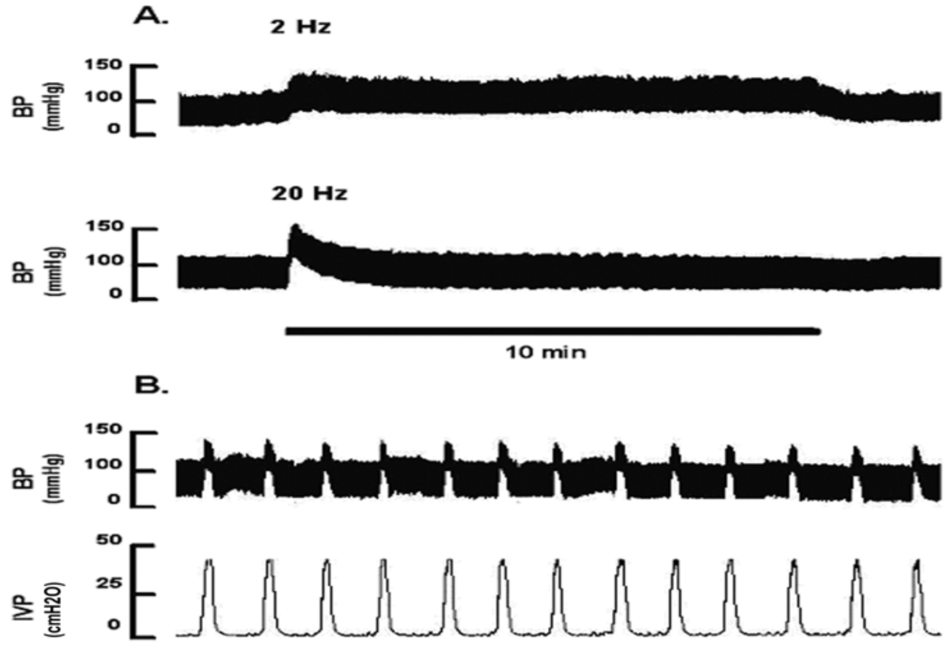
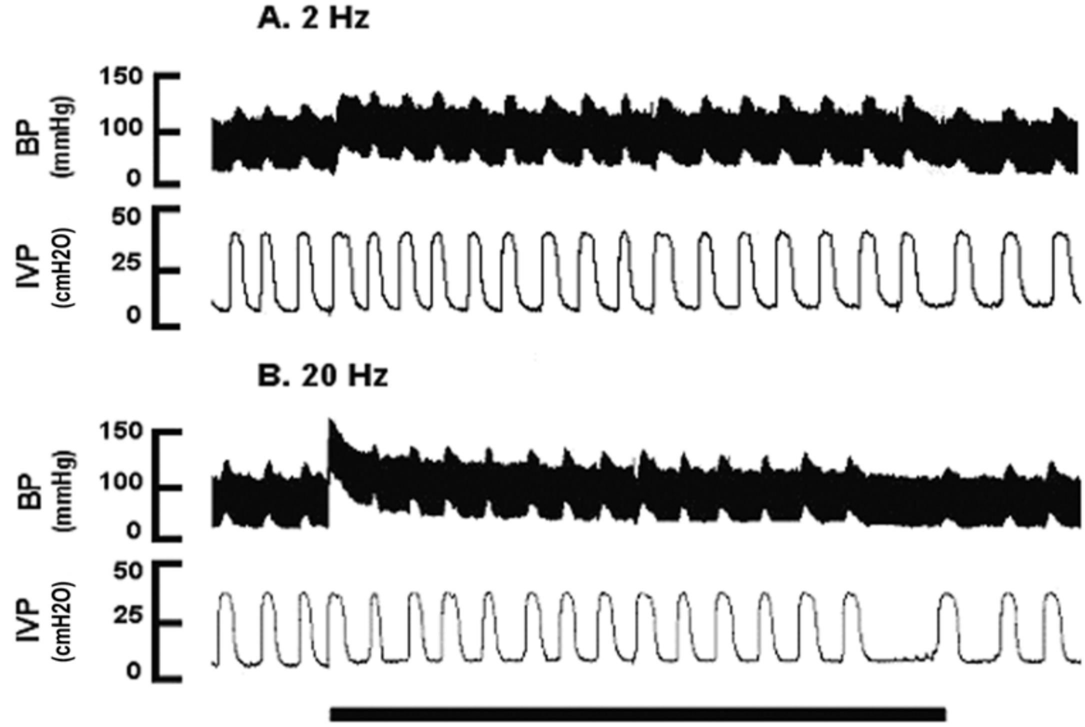

A.
2 Hz
150
100
0
BP (mmHg)
20 Hz
150
100
0
BP (mmHg)
10 min
B.
150
100
0
BP (mmHg)
50
25
0
IVP (cmH2O)

Figure 1



A. 2 Hz
BP (mmHg)
150
100
0
IVP (cmH2O)
50
25
0
B. 20 Hz
BP (mmHg)
150
100
0
IVP (cmH2O)
50
25
0

Figure 2

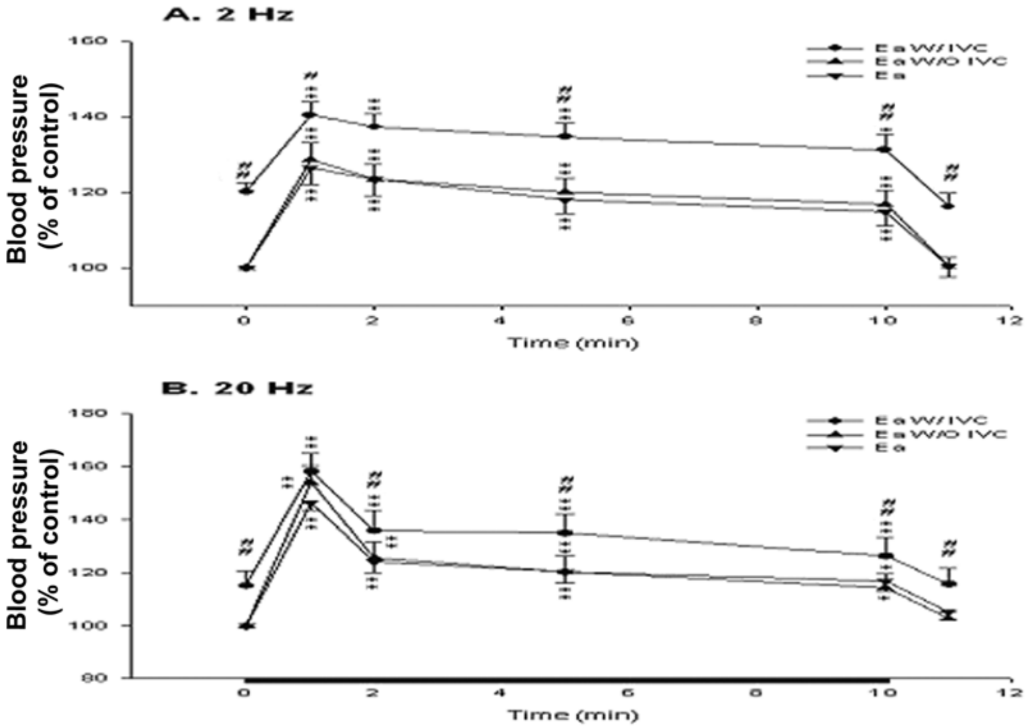
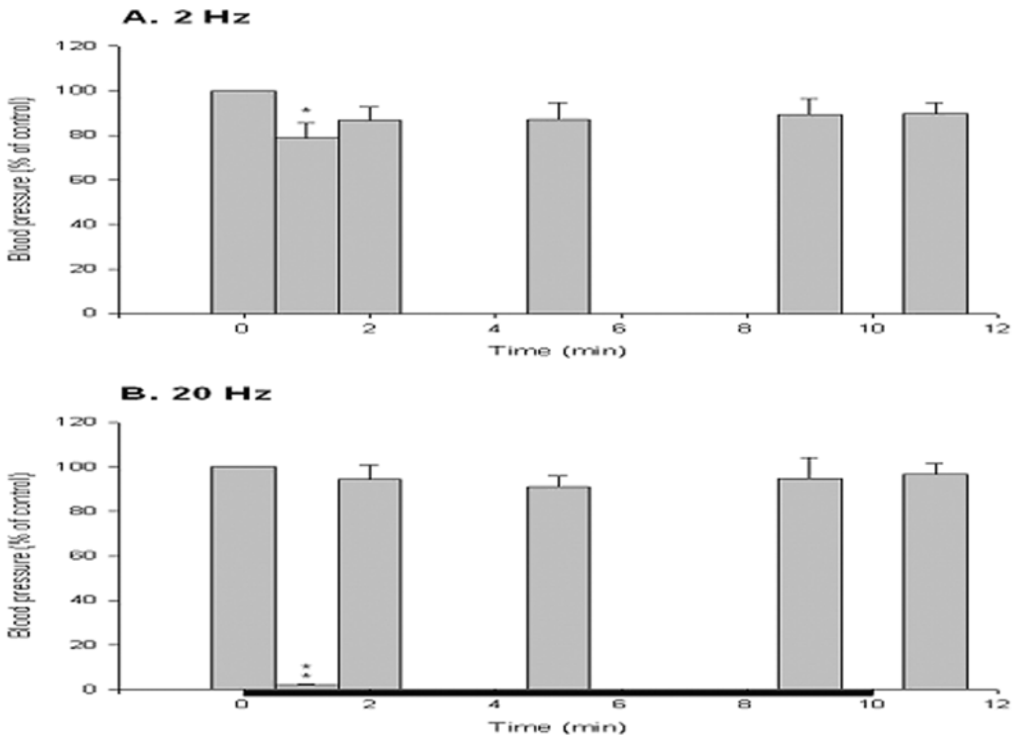

A. 2 Hz
Blood pressure (% of control)
Time (min)
Ea W/ IVC
Ea W/O IVC
Ea
B. 20 Hz
Blood pressure (% of control)
Time (min)
Ea W/ IVC
Ea W/O IVC
Ea

Figure 3



A. 2 Hz
Blood pressure (% of control)
120
100
80
60
40
20
0
*
0
2
4
6
8
10
12
Time (min)
B. 20 Hz
Blood pressure (% of control)
120
100
80
60
40
20
0
**
0
2
4
6
8
10
12
Time (min)

Figure 4