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Effect of electroacupuncture on response to immobilization stress

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Abstract

Forced immobilization is a simple and effective stressor which produces large increases in heart rate (HR), blood pressure (BP), and plasma levels of norepinephrine (NE) and epinephrine (EPI). This study investigated the effects of electroacupuncture on BP, HR, and plasma catecholamine levels in rats challenged with immobilization stress. Male Sprague–Dawley rats received electroacupuncture (3 Hz, 0.2 ms pulses, 20 mA) for 30 min after start of immobilization stress (180 min). Needlepoints corresponded to Shaohai (HT3) and Neiguan (PC6) on the heart and pericardium channel. BP and HR were monitored with an indwelling carotid catheter, and blood samples were taken from the jugular vein. Blood (for HPLC determination of NE and EPI), mean BP, and HR were sampled at rest and during the immobilization stress at 15, 30, 60, 90, 120, 150, and 180 min. Electroacupuncture at HT3 and PC6 points but not at control points (TE5, LI11, and tail) significantly reduced the expected increases in BP, HR, and attenuated plasma levels of NE and EPI in response to 3 h of immobilization stress. Results provide strong evidence that electroacupuncture effectively reduces BP and HR increases and plasma catecholamine increases in rats challenged with immobilization stress.

Keywords: Acupuncture; Current intensity; Epinephrine; Sympathetic nerve; Heart rate; Catecholamines; Stress reaction; Sympathoneural; Blood pressure; Norepinephrine; Yin and Yang; Adrenomedullary

1. Introduction

Oriental medicine is largely descriptive and philosophical rather than quantitative. The human body is considered to be a complex network of intricately related processes played upon by opposing forces (Yin and Yang). The two forces always combine to make up the whole. This polar system has an important role in the description of life processes in the human body and of their disturbance. Health is viewed as the maintenance of harmony between Yin and Yang similar to the autonomic nervous system with its duality of sympathetic versus parasympathetic nervous system, while illness is an expression of disharmony. If one system outweighs the other, abnormal symptoms are felt by the patient depending upon which side is excessive and which is deficient (Dale, 1982; Stux and Pomeranz, 1987).

Acupuncture plays a role in manipulating and balancing Yin and Yang when the body's innate homeostatic potentialities are overwhelmed by acute or chronic stress conditions (Dale, 1982). Acupuncture is essentially a technique for correcting reversible physiological malfunction of various parts of the body by physiological means. Acupuncture-initiated impulses may activate the autonomic centers and the hypophyseal system in the brain so as to improve the efficiency of homeostatic and self-defense mechanisms of the body (Chang, 1982). Results of some animal and clinical studies provide evidence for the involvement of the autonomic nervous system and endocrine system in the action of acupuncture (Cao et al., 1983; Ionescu-Tirgoviste et al., 1991; Ku and Zou, 1993).

Acupuncture as a therapeutic intervention has been widely practiced in eastern countries for thousands of years. Although introduced relatively recently, acceptance of acupuncture by the general public has increased rapidly in

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western countries. The discovery of the central endorphin system was a prominent step toward understanding the analgesia effect of acupuncture and many studies have been undertaken to investigate the mechanisms of acupuncture analgesia. Studies in laboratory animals and humans have demonstrated that acupuncture can cause multiple biological responses that occur locally and at a distance (Filshie and White, 1998). Most studies have been published only in the Chinese language and there are still many open questions.

Few experiments have investigated the effect of acupuncture on stress response. These stress-related studies, using animal models, have provided evidence that acupuncture can reduce norepinephrine (NE) levels in perfusate of brain regions as well as in the circulating blood (Cao et al., 1983), reduce secretion of adrenal hormones in animals exposed to immobilization stress (Liao et al., 1980), induce long-lasting cardiovascular and behavioral depression in spontaneous hypertensive rats (SHR) (Yao et al., 1982a), and produce an anxiolytic effect in animals exposed to restraint-induced stress (Guimaraes et al., 1997).

Physical and psychological stressors are known to cause a wide variety of behavioral and biochemical alterations in organisms including effects on cardiac functions, blood pressure (BP), and systemic release of catecholamines (Chrousos and Gold, 1992; Clow et al., 1998). Forced immobilization has been shown to be a simple and effective physiological stressor that produces large increases in heart rate (HR), BP, and plasma levels of NE and epinephrine (EPI), by activation of the sympatho-adrenal medullary system and pituitary–adrenocortical axis. These effects have been confirmed in a variety of experimental studies (Chiueh and Kopin, 1978; Kventnansky et al., 1979; Eikenburg, 1992; Kvetnansky et al., 1992; Gomez et al., 1996).

In oriental medicine, the heart and pericardium (Yin) channels work as a functional unit and are associated with the brain and its mental functions (Stux and Pomeranz, 1987, 1988). Shaohai (HT3) and Neguan (PC6) on the heart and pericardium channel are points that are frequently used to treat mental, psychiatric, and psychosomatic disorders and are known clinically to produce a sedation effect (Stux and Pomeranz, 1987, 1988). On the contrary, Quchi (LI11) and Waiguan (TE5) on the large intestine and triple energizer (Yang) channels are effective points that treat the depressed immune system and pain/polyneuropathy of the arm, respectively (Stux and Pomeranz, 1987, 1988).

The present study was carried out to investigate the effects of electroacupuncture on BP, HR, and plasma catecholamine levels in rats challenged with immobilization stress and to identify the sedation/anxiolytic effects of electroacupuncture at Shaohai (HT3) and Neiguan (PC6) compared to electroacupuncture at Quchi (LI11) and Waiguan (TE5) and a nonacupuncture point.

2. Method

2.1. Subjects

Subjects were male Sprague–Dawley rats, weighing between 300 and 350 g at the start of the experiment. Rats were obtained from Daehan Animal (Seoul, Korea/SPF animals). All rats were kept on ad libitum food and water and maintained on a 12 h light–dark cycle throughout the course of the study. The experiments reported here were approved by the Coatesville VAMC Institutional Animal Care and Use Committee.

2.2. Surgery

Rats were anesthetized with sodium pentobarbital (50 mg/kg body weight ip) A PE-50 polyethylene catheter was implanted in the aortic arch via the left common carotid artery to monitor arterial BP and HR. A PE-50 catheter was put into the right external jugular vein for collection of plasma samples for catecholamine determinations in separate groups of animals. Catheters were plugged and exteriorized via a skin incision in the back of the neck. Patency of the catheter was maintained by twice-daily flushes of 0.5-ml saline containing 500 IU heparin. Rats were exposed to immobilization stress 48 h after recovery from surgery. Rats, wearing an animal jacket, were immobilized for 180 min to induce immobilization stress by taping all four limbs in a specially prepared immobilization frame.

2.3. Determination of BP and HR

Mean arterial BP was recorded on one channel of a polygraph (Grass Model 7D) via a P23DC transducer. HR was recorded using cardiotachograph (Grass model 7P44C) triggered by the arterial pulse. BP and HR were expressed using the mean of 10 samples taken from a 5-min-long graphic recording at each sample time (an interval of 30 s between samples). BP and HR samples were obtained at rest and during the immobilization stress at 15, 30, 60, 90, 120, 150, and 180 min.

2.4. Measurement of plasma catecholamines

Blood samples (0.6 ml) were taken before (time 0, prestress baseline) and during the immobilization stress at 15, 30, 60, 120, and 180 min (Kventnansky et al., 1979). After each blood withdrawal, an equal volume of sterile heparinized (100 IU/ml) physiological saline was injected. All blood samples were immediately centrifuged ($3000 \times g$) at 4 °C for 10 min and plasma was stored at -70 °C until assayed. Plasma concentrations of catecholamines (NE and EPI) were assayed using HPLC with electrochemical detection and internal standard method after a modified batch alumina extraction as described by others (Holmes et al., 1994). The HPLC system consisted of an isocratic pump

(Waters 510), Waters 460 Amperometric Detector, and Spherisorb ODS2 (150 × 4.6 mm, 5 μM column). The working electrode for the detection was set at 630 mA. The composition of the mobile phase was 75 mM potassium phosphate monobasic, 1.4 mM sodium octanesulfonate, 10 M EDTA, and 5% acetonitrile, pH 3.3.

2.5. Electroacupuncture and immobilization stress procedure

Electroacupuncture was given at points corresponding to bilateral Shaohai (HT3) and Neiguan (PC6), bilateral Quchi (LI11) and Waiguan (TE5) and tail (nonacupoint electrical stimulation supine control). All points were stimulated with a Grass Stimulator (Model G880) using rectangular pulses with a pulse duration of 0.2 ms, a frequency of 3 Hz, and an intensity of 20 mA, for 30 min after start of immobilization stress (180 min). Stainless-steel needles with a diameter of 0.18 mm and a length of 20 mm were inserted vertically to a depth of 3 mm into acupoints and nonacupoints. These points were also stimulated using current intensities of 2.5, 5, and 10 mA in separate groups of animals ($n = 7$) to investigate the effect of electroacupuncture current intensity on the stress response. Anatomical location of stimulated acupoints were those corresponding to acupoints in man as described by Stux and Pomeranz (1987) and in animal acupuncture atlases (Klide and Kung, 1977; Lee, 1983; Schone, 1999). The supine position was adopted to expose acupuncture points corresponding to HT3 and PC6 during the immobilization stress. The tail was used as a nonacupoint electrical stimulation control site (Han et al., 1999). Needles were placed into nonacupoints 0.2 (1/5 tail length) from the proximal region of the tail to avoid the two tail acupoints (proximal tail and tip of the tail). These nonacupoints are distal to the proximal tail acupoints. The prone position was adopted to expose acupuncture points, LI11 and TE5, during the immobilization stress. Since immobilization in the prone position could be differentially stressful than immobilization in the supine position, the prone position was used as a control for electroacupuncture at LI11 and TE5 (see Fig. 1).

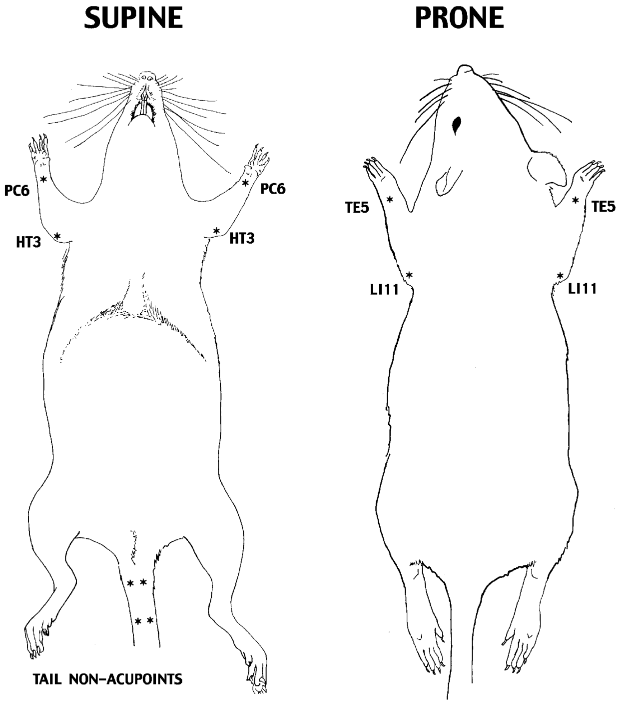

Fig. 1. Diagram of rat in supine and prone positions with acupuncture points HT3 (Shaohai), PC6 (Neiguan), LI11 (Quchi), TE5 (Waiguan), and nonacupuncture tail stimulation points shown. Animals receiving electrical stimulation at HT3–PC6 acupoints and tail nonacupoints were immobilized in the supine position. Animals receiving stimulation at LI11–TE5 acupoints were immobilized in the prone position.

Rats (HT3–PC6 group) were immobilized in the supine position to expose acupuncture points corresponding to Shaohai (HT3) and Neiguan (PC6) and tail nonacupoints during immobilization stress. The tail was used as an electrical stimulation control site in some animals (nonacupoint electrical stimulation supine group) to determine the effects of electrical stimulation at a nonacupuncture point during immobilization stress in the supine position. Rats (LI11–TE5 group) were placed in the prone position to expose acupuncture points, Quchi (LI11) and Waiguan (TE5) during immobilization stress (Fig. 1). To control for the possibility that the stress response to immobilization in the supine position might be greater than immobilization in the prone position, two additional groups of animals (prone control group and supine control group) were exposed to immobilization stress without insertion of needles or electrical stimulation. The prone control group was used as a control for electroacupuncture at Quchi (LI11) and Waiguan (TE6) and the supine control group was used as a control for electroacupuncture at Shaohai (HT3) and Neiguan (PC6). The tail (nonacupoint electrical stimulation group), immobilized in the supine position, was used as a control for the HT3–PC6 group, which was also immobilized in the supine position.

2.6. Statistical analysis

Statistical analysis of data was carried out using the SPSS 8.0 and Statview 5.0 software programs. Serial samples of HR, BP, and plasma catecholamines values were statistically analyzed by repeated-measures ANOVAs and post hoc Tukey tests to compare the experimental and control groups. Pearson regression analysis was used to determine if there was a significant relationship between current intensity and the HR, BP, and plasma catecholamines values measured after 180 min of immobilization stress in the HT3–PC6 group.

3. Results

3.1. Heart rate

All groups demonstrated elevated HRs ranging from 120% to 152% above baseline resting HR when exposed

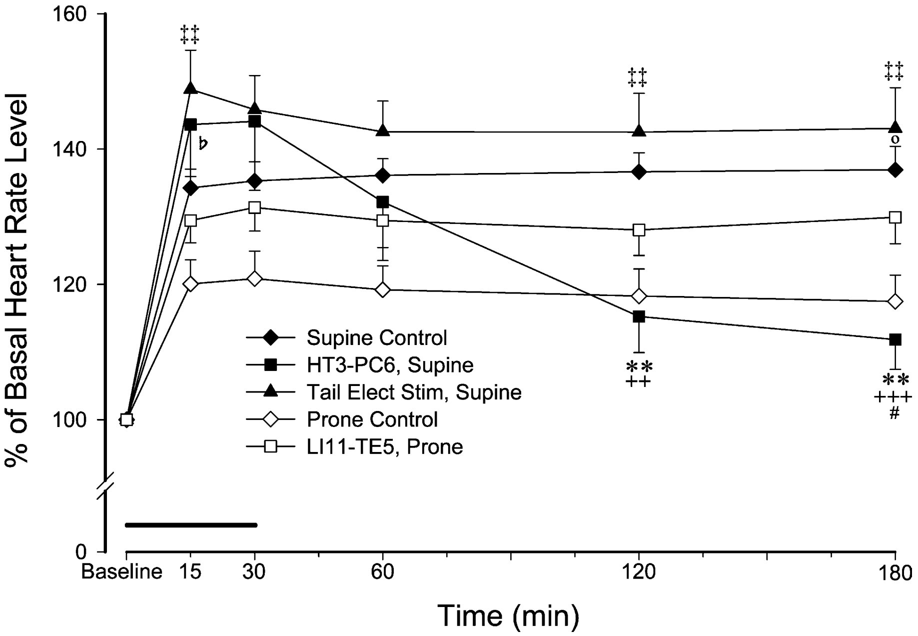

Fig. 2. Effect of electroacupuncture on HR (mean ± S.E.M. percent of baseline) of male Sprague–Dawley rats challenged with immobilization stress for 180 min and given electroacupuncture (3 Hz, 0.2 ms pulses, 20 mA) for the first 30 min after start of immobilization stress. The horizontal bar indicates the duration of electroacupuncture. HT3–PC6 group, $n=10$; LI11–TE5 group, $n=8$; tail electrical stimulation group, $n=8$; prone control group, $n=7$; supine control group, $n=10$; repeated ANOVA and post hoc Tukey test. $^{**}P<.01$, HT3–PC6 group vs. supine control group; $^{++}P<.01$, $^{+++}P<.001$, HT3–PC6 group vs. tail electrical stimulation group; $^{\#}P<.05$, HT3–PC6 group vs. LI11–TE5 group; $^{b}P<.05$, HT3–PC6 group vs. prone control group; $^{‡‡}P<.01$, tail electrical stimulation group vs. prone control group; $^{o}P<.05$, supine control group vs. prone control group.

to immobilization stress. HRs remained elevated in the LI11–TE5 group and all control groups throughout the 180 min of immobilization stress (Fig. 2). Control animals immobilized in the supine position had significantly ($P<.05$, 180 min) higher HRs than control animals immobilized in the prone position, while control animals immo-

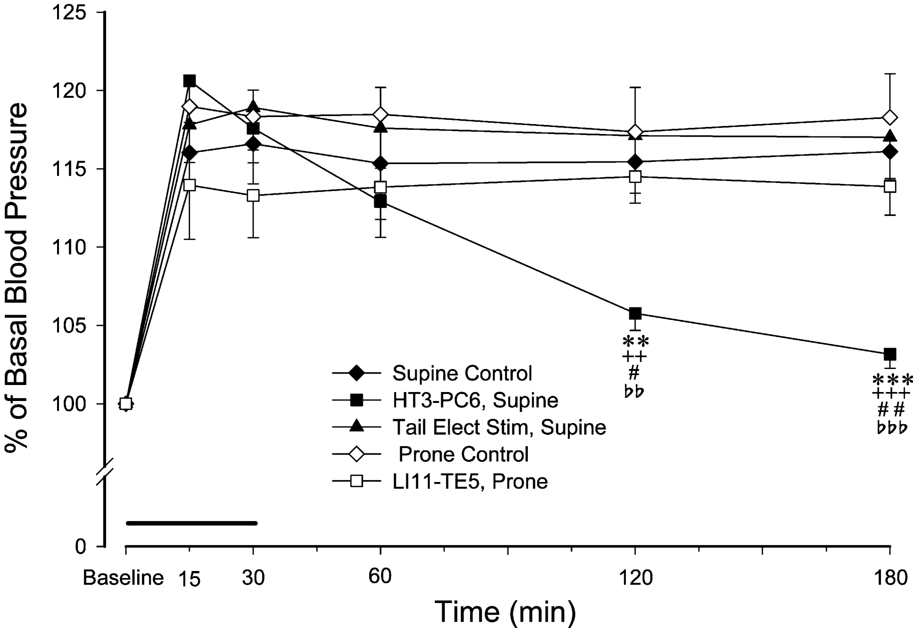

Fig. 3. Effect of electroacupuncture on BP (mean ± S.E.M. percent of baseline) of male Sprague–Dawley rats challenged with immobilization stress for 180 min and given electroacupuncture (3 Hz, 0.2 ms pulses, 20 mA) for the first 30 min after start of immobilization stress. The horizontal bar indicates the duration of electroacupuncture. HT3–PC6 group, $n=10$; LI11–TE5 group, $n=8$; tail electrical stimulation group, $n=8$; prone control group, $n=7$; supine control group, $n=10$; repeated ANOVA and post hoc Tukey test. $^{**}P<.01$, HT3–PC6 group vs. supine control group, $^{***}P<.001$, HT3–PC6 group vs. supine control group; $^{++}P<.01$, $^{+++}P<.001$, HT3–PC6 group vs. tail electrical stimulation group; $^{\#}P<.05$, $^{\#\#}P<.01$, HT3–PC6 group vs. LI11–TE5 group; $^{bb}P<.01$, $^{bbb}P<.001$, HT3–PC6 group vs. prone control group.

bilized and receiving electrical stimulation of the tail had the highest HRs. Animals in the HT3–PC6 group showed elevated HRs for the first 30 min of immobilization stress during which electroacupuncture was applied bilaterally. However, the HT3–PC6 group showed a marked decrease in HR after termination of electroacupuncture when measured at 60, 120, and 180 min (Fig. 2). Group LI11–TE5 animals tended to have greater HRs than the prone control group, but these differences were not statistically significant.

3.2. Blood pressure

Similar trends were demonstrated for BP with all groups showing elevated BP in response to immobilization stress. BP remained elevated above baseline levels in the LI11–TE5 group and all control groups throughout the 180 min of immobilization stress. Although not statistically significant, the LI11–TE5 group showed the smallest and the prone control group the greatest maintained increase in BP, respectively (Fig. 3). Control animals receiving electrical stimulation of the tail and control animals restrained in the supine position had similar BP elevations. Animals in the HT3–PC6 group had their greatest BP increase at 15 min followed by highly significant decreases in BP between 30 and 180 min (Fig. 3). After 180 min of immobilization stress, the HT3–PC6 group's BP was not significantly different from baseline prestress resting BP.

3.3. Plasma catecholamines

3.3.1. Norepinephrine

When exposed to immobilization stress, all groups demonstrated elevated plasma NE levels ranging from 225% (prone control group) to 500% (tail electrical stimulation supine group) above baseline resting plasma levels. During immobilization stress, NE levels remained elevated above baseline levels in all groups with the exception of the HT3–PC6 group which demonstrated highly significant decreases in NE levels starting at 30 min and continuing to the end of the immobilization stress period at 180 min (Fig. 4). After 180 min of immobilization stress, the HT3–PC6 group NE levels were near baseline prestress resting NE plasma levels.

3.3.2. Epinephrine

Similar trends were demonstrated for EPI with all groups showing elevated plasma NE levels ranging from 220% to 250% (LI11–TE5 and prone control groups) to 900% (tail electrical stimulation supine group) above baseline resting plasma levels. Similar to the NE data, only the HT3–PC6 group showed significant decreases in EPI plasma levels following electroacupuncture (Fig. 5).

3.4. Current intensity

As seen in Fig. 6, there was a strong statistically significant ($P<.001$) relationship between the current

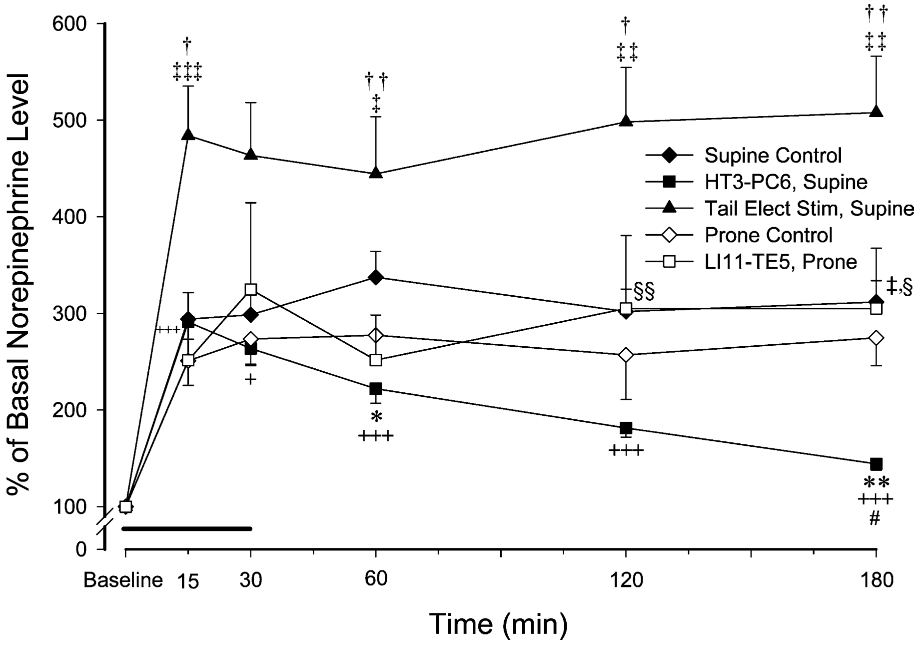

Fig. 4. Effect of electroacupuncture on plasma NE levels (mean ± S.E.M. percent of baseline) of male Sprague–Dawley rats challenged with immobilization stress for 180 min and given electroacupuncture (3 Hz, 0.2 ms pulses, 20 mA) for the first 30 min after start of immobilization stress. The horizontal bar indicates the duration of electroacupuncture. HT3–PC6 group, $n=17$; LI11–TE5 group, $n=10$; tail electrical stimulation group, $n=10$; prone control group, $n=10$; supine control group, $n=17$; repeated ANOVA and post hoc Tukey test. $^{*}P<.05$, $^{**}P<.01$, HT3–PC6 group vs. supine control group; $^{+}P<.05$, $^{+++}P<.001$, HT3–PC6 group vs. tail electrical stimulation group; $^{\#}P<.05$, HT3–PC6 group vs. LI11–TE5 group; $^{\ddagger}P<.05$, $^{\ddagger\ddagger}P<.01$, $^{\ddagger\ddagger\ddagger}P<.001$, tail electrical stimulation group vs. prone control group; $^{\dagger}P<.05$, $^{\dagger\dagger}P<.01$, tail electrical stimulation group vs. LI11–TE5 group; $\ddagger P<.05$, supine control group vs. LI11–TE5 group; $^{\S}P<.05$, $^{\S\S}P<.01$, supine control group vs. tail electrical stimulation group.

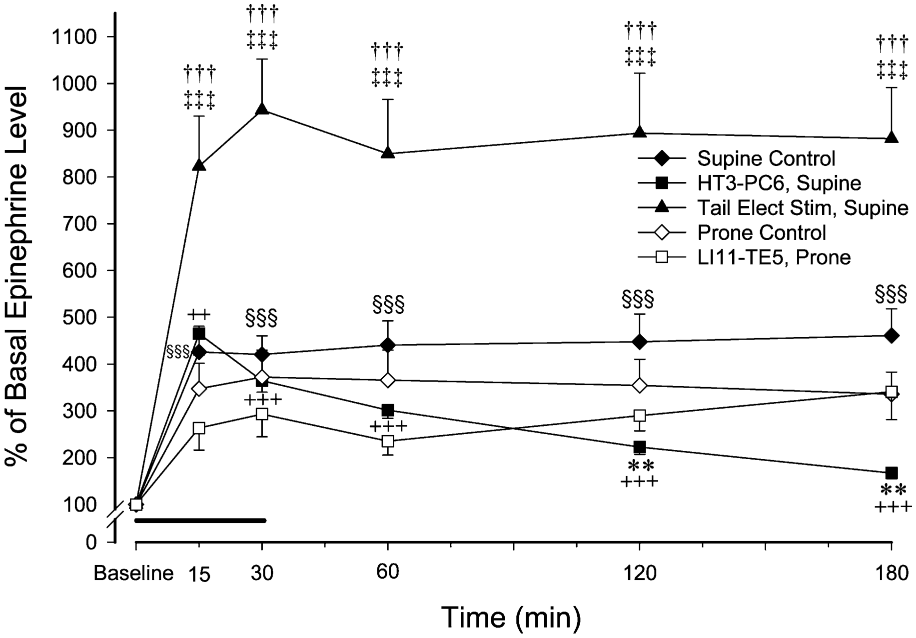

Fig. 5. Effect of electroacupuncture on plasma EPI levels (mean ± S.E.M. percent of baseline) of male Sprague–Dawley rats challenged with immobilization stress for 180 min and given electroacupuncture (3 Hz, 0.2 ms pulses, 20 mA) for the first 30 min after start of immobilization stress. The horizontal bar indicates the duration of electroacupuncture. HT3–PC6 group, $n = 17$; LI11–TE5 group, $n = 10$; tail electrical stimulation group, $n = 10$; prone control group, $n = 10$; supine control group, $n = 17$; repeated ANOVA and post hoc Tukey test. $^{**}P<.01$; $^{++}P<.01$, $^{+++}P<.001$, HT3–PC6 group vs. tail electrical stimulation group; $^{\ddagger\ddagger\ddagger}P<.001$, tail electrical stimulation group vs. prone control group; $^{\dagger\dagger\dagger}P<.001$, tail electrical stimulation group vs. LI11–TE5 group; $^{\S\S\S}P<.001$, supine control group vs. tail electrical stimulation group.

intensity applied to HT3–PC6 acupoints and all physiological indices of stress with higher currents intensities producing greater decreases in HR, BP, plasma NE and EPI when measured at 180 min of the immobilization stress. Pearson correlation coefficients were $r = -.68$ HR, $r = -.73$ BP, $r = -.68$ NE, $r = -.63$ EPI.

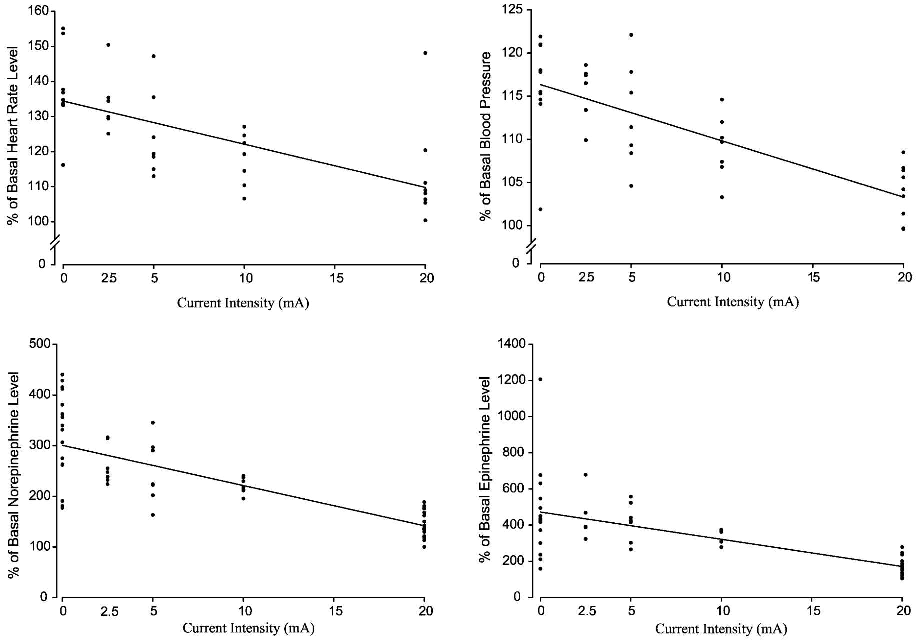

Fig. 6. Effect of increasing current intensities at HT3–PC6 acupoints on HR, BP, and plasma catecholamine levels (NE and EPI) shown as group mean percent of baseline in male Sprague–Dawley rats challenged with immobilization stress for 180 min. Separate groups of rats were given electroacupuncture (3 Hz, 0.2 ms pulses, for the first 30 min after start of immobilization stress) at current intensities of 0 mA ($n = 10$), 2.5 mA ($n = 7$), 5 mA ($n = 7$), 10 mA ($n = 7$), 20 mA ($n = 10$). Data points (shown as percent of prestress values) are after 180 min of immobilization stress, $P<.001$, HR—$r = -.68$, BP—$r = -.73$, NE—$r = -.68$, EPI—$r = -.63$.

4. Discussion

The term "stress reaction" is often used to refer to an organism's response to challenges that disturb psychological and/or physiological homeostasis. Stress acts as a warning signal, generated in the brain, detecting danger in the environment and indicating that an immediate action is required. When the organism's regulatory mechanisms fail to function in a timely and appropriate manner, abnormal pathological conditions that interfere with a healthy well-being become evident. The hallmarks of the stress reaction are activation of the sympathetic–adrenal medullary system and the hypothalamic–pituitary–adrenal axis (Clow et al., 1998). Acute stressors increase plasma levels of catecholamines and peak catecholamine levels increase, in a stepwise fashion, with increasing magnitude of the stressor (Natelson al., 1981; Goldsteine et al., 1983). Plasma NE and EPI levels have been shown to correlate positively with (systolic and diastolic) BP and HR during operant BP conditioning in baboons (Goldsteine et al., 1981). It has been suggested that changing levels of plasma NE during stress primarily reflect sympathetic nervous outflow, whereas plasma EPI levels derive from adrenal medullary secretion (Axelrod and Reisine, 1984). These studies show an association between sympathetic activity and BP and between adrenomedullary activity and HR.

Forced immobilization stress of animals increases HR, BP, and plasma catecholamines (Kventnansky et al., 1979; Gomez et al., 1996). These physiological indices of stress are associated with behavioral indices of stress such as increased vocalization, struggling, increased defecation and urination (Deturk and Yoge, 1982). In our experiments, we investigated the effects of electroacupuncture on immobilization stress by stimulating Shaohai (HT3) and Neiguan (PC6) on the heart and pericardium channel which are known clinically to produce a sedation effect. This was compared with the effects of electroacupuncture at Quichi (LI11) and Waiguan (TE5) acupoints that are used clinically to treat depression. We controlled for nonspecific effects of electrical stimulation by electrical stimulation of a nonacupoint (tail electrical stimulation supine control group) with animals immobilized in the supine position. Two groups of nonelectrically stimulated animals (supine and prone control groups) were also subjected to forced immobilization in an attempt to control for the effects of body position on immobilization stress responses.

The present results demonstrated a significant increase in BP, HR, and plasma levels of catecholamine that were sustained during a 3-h immobilization stress period. These changes are consistent with previous findings (Kventnansky et al., 1979; Eikenburg, 1992). Electroacupuncture at HT3 and PC6 points, but not supine control nor electrical tail stimulation in the supine position, significantly reduced the expected increases in BP, HR, and attenuated the increase in plasma levels of NE and EPI in response to 3 h of immobilization stress. The attenuated stress responses in the HT3 and PC6 acupoints group occurred only after termination of the electroacupuncture. However, rats receiving electroacupuncture at LI11 and TE5 did not show a significant difference in stress response from rats immobilized in the prone position. Electroacupuncture at HT3 and PC6 points produced an intensity-dependent decrease in immobilization stress-induced HR, BP, and plasma catecholamine responses (Fig. 6).

Immobilization in the supine position produced higher HRs than immobilization in the prone position. Thus, in terms of cardiac response, immobilization in the supine position appears to be a greater stressor than immobilization in the prone position. Rats (supine control) subjected to immobilization stress for 3 h in a supine position showed HR and BP increases of 30% and 15%, respectively. These results agree with reports that immobilized rats exhibit significant increases in HR and BP (Chiueh and Kopin, 1978; Kventnansky et al., 1979) and that a single 2.5-h stress period produces significant increases in BP, HR, and plasma catecholamines (Eikenburg, 1992).

The anatomical location of acupoints for the HT3–PC6 group were those corresponding to Shaohai (HT3) and Neiguan (PC6) in man on both arms (see Fig. 1). These points were stimulated electrically for the first 30 min of the immobilization stress period. During the period of electroacupuncture stimulation, the HT3–PC6 group had greater HR and BP increases than the supine control group. However, after 30 min, the HR and BP of the HT3–PC6 group began to decrease and was significantly lower after 1.5 h, approaching baseline resting values by 3 h. Yao showed that prolonged low-frequency electroacupuncture-like stimulation (3 Hz, 0.2 ms pulses) applied to the sciatic nerve induced a long-lasting (up to 12 h) depressor response and bradycardia in unanesthetized SHR and Wistar–Kyoto normotensive rats. Yao found that these effects were obtained only if electrical stimulation was sufficiently strong to produce muscle contraction. The stimulation parameters used in Yao's study are similar to those of electroacupuncture stimulation that give rise to the clinical sensation of swelling and numbness (Teh Chi) which many acupuncturists believe to be essential to obtain effective acupuncture treatment (Yao et al., 1982a,b). In our study, HT3 and PC6 were stimulated with a pulse duration of 0.2 ms, a frequency of 3 Hz, and a current intensity of 20 mA. Current intensities between 2.5 and 20 mA were strong enough to produce muscle twitch/contraction. HR and BP in all other groups including the LI11–TE5 group remained elevated for the entire 3 h of immobilization stress. Animals receiving electrical stimulation of the tail (electrical stimulation supine control group) showed the greatest increase in HR while exhibiting BP elevations similar to the supine control group. These results suggest that electrical stimulation of the tail increases the stress response but electroacupuncture at Shaohai (HT3) and Neiguan (PC6) reduces the stress response. Waiguan (TE5) and Quchi (LI11) acupoints are on the triple energizer channel and large intestine channel on the dorsal side of the arm (see Fig. 1). These acupoints are directly opposite the Neiguan (PC6) and Shaohai (HT3) acupoints, respectively, and were chosen for comparison with Shaohai (HT3) and Neiguan (PC6) stimulated animals and nonstimulated animals immobilized in the prone position. We compared the LI11–TE5 group with the prone control group because animals were placed in the prone position to electrically stimulate Waiguan (TE5) and Quchi (LI11) acupoints. There were no significant differences in HR or BP between the LI11–TE5 group and the prone control group, although there was a trend for the LI11–TE5 group to have higher HR and lower BP than the prone control group throughout the entire 3-h immobilization stress period. Our results suggest that electroacupuncture at Waiguan (TE5) and Quchi (LI11) has a slight effect on the immobilization stress reaction.

Forced immobilization of rats increases plasma level of CAs which in turn are associated with increases in BP and HR (Kventnansky et al., 1979). One study attempted to determine the origin of the increased plasma levels of EPI and NE in rats exposed to forced immobilization by using adrenal medullectomy and chronic guanethidine treatment (Kventnansky et al., 1979). This study showed that immobilization stress-induced increases in plasma NE was due to increased sympathetic nerve activity while increased plasma EPI was due to increased adrenal medulla activity (Kventnansky et al., 1979). In our study, in rats (HT3–PC6 group) receiving electroacupuncture at Shaohai (HT3) and Neiguan (PC6), NE and EPI plasma levels began to markedly decrease after 15 min and declined to resting prestress levels by 3 h of immobilization stress. This is compared to the supine control group and the electrical stimulation supine control group, both of which maintained elevated NE and EPI plasma levels throughout the 3 h of immobilization stress. Plasma levels of NE and EPI were elevated and remained elevated for the LI11–TE5 group and all control groups. The tail electrical stimulation supine control group exhibited the greatest sustained elevations of both NE and EPI. Plasma levels of NE and EPI in the tail electrical stimulation supine group were significantly increased compared with electroacupuncture-treated groups and the supine and prone control groups. This suggests that (simple) electrical stimulation at a nonacupoint increases the stress reaction. There was little change in plasma levels of NE or EPI in the Waiguan (TE5) and Quchi (LI11) group compared to the prone control group. Our results are similar to others who showed that electroacupuncture can reduce stress responses such as increased levels of the plasma catecholamines NE, dopamine, corticosterone, and ACTH after tooth-pulp stimulation in anesthetized rats (Han et al., 1999). However, our results differ with an earlier study which showed that acupuncture did not produce an effect on cardiovascular or hormonal responses in restraint-stressed rats; yet produced an acupuncture-induced anxiolytic effect as demonstrated by a decrease in stress-related behaviors (Guimaraes et al., 1997). Such inconsistencies may be at least in part related to use of different acupuncture points and/or differences in acupuncture methods (manual stimulation vs. electrical stimulation).

Our results suggest that electroacupuncture at Shaohai (HT3) and Neiguan (PC6) has a strong inhibitory effect on sympathetic nerve and possibly adrenal medullary activity in rats subjected to immobilization stress and that this effect is specific to the Shaohai (HT3) and Neiguan (PC6) acupoints. Furthermore, there appears to be a strong inverse correlation between current intensity at Shaohai (HT3) and Neiguan (PC6) acupoints and degree of stress reduction (Fig. 6). One possible mechanism where stimulation of specific Yin acupoints with electroacupuncture could diminish the HR and BP changes seen during immobilization stress is by affecting plasma or brain levels of catecholamines. Other studies have reported that forced immobilization stress facilitates the release of NE in the paraventricular nucleus of the hypothalamus and suggest that the increases in HR and BP are mediated by activation of the hypothalamo–pituitary–adrenal system (Nakata et al., 1993; Pacak et al., 1995). Determination of the specific mechanisms involved in electroacupuncture-induced stress reduction will require additional study.

In conclusion, our results provide strong evidence that electroacupuncture at Shahai (HT3) and Neiguan (PC6) acupoints effectively reduced BP and HR increases and plasma catecholamine increases in rats challenged with immobilization stress. Our results suggest that the reduced stress response produced by electroacupuncture is most likely mediated via an inhibition of the sympatho-adrenal medullary system as revealed by the parallel decreases of cardiovascular and neurochemical responses to immobilization stress. In addition, the results of these experiments are consistent with the Yin meridian having a sedation effect in oriental acupuncture and suggests that the Yin meridian has a different physiological effect in maintaining homeostasis compared with the Yang meridian.

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