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Research report

Protective effects of electroacupuncture on cardiac function in rats subjected to thoracic surgery trauma

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ABSTRACT

The present study investigates the protective effects of electroacupuncture (EA) application on cardiac function, while simultaneously exploring the underlying neurobiological mechanisms, in rats that have experienced thoracic surgery-induced stress. Mean arterial and left intraventricular pressures were monitored as indicators of cardiac function. Meanwhile, the immunohistochemistry for c-Fos protein expression and electrophysiology in vitro in brain nuclei, known to regulate cardiac function, provide insights into the effects of EA on the central nervous system. The results show that cardiac function was dramatically suppressed with thoracic surgery trauma, the expression levels of c-Fos in the paraventricular nucleus (PVN) and the rostral ventrolateral medulla (RVLM) significantly increased, the rheobase intensity of the intracellular current injection needed to initiate the action potential decreased, membrane resistance in the PVN neurons significantly increased, and the inductivity of the postsynaptic potentials in the PVN neurons of the surgery-treated rats significantly decreased. EA application at the Neiguan acupoints (PC6) attenuated the decreases in almost all investigated functional parameters of the heart. EA significantly decreased the number of Fos-immunoreactive neurons in the PVN and RVLM, significantly decreased the Max L. slope of the PVN neurons, and increased the inductivity of the postsynaptic potentials in the PVN neurons of the surgery-treated rats. These data indicate the protective effects of EA application on cardiac function in rats that have experienced surgery-induced stress and show that EA application at the Neiguan acupoints may produce its protective effects through the neurons in the PVN and the RVLM.

1. Introduction

Surgical trauma is a stressor and may provoke sudden cardiovascular complications (e.g. myocardial infarction, cardiac arrest, unstable angina, unstable cardiac rhythms, and ischemic/thrombotic stroke) (London, 2009; Sirieix et al., 1998). Surgical trauma-induced stress responses are associated with organ failure, tissue catabolism, and prolongation of recovery in surgical patients (Wilmore, 2002). Therefore, finding stress-reduction techniques that contribute to reduced complications, shortened length of convalescent recovery, and greatly improved operative outcomes is necessary.

Acupuncture has been widely used as a therapeutic intervention in clinical medicine in humans and animals (Chan et al., 2001; Lin et al., 2003, 2008; Park et al., 2001), and has shown enhancing effects on cardiac function in cardiovascular diseases (Ballegaard et al., 1993; Li et al., 1998; Longhurst, 2007; Richter et al., 1991; Wang et al., 2003; Xia et al., 2008). Ballegaard et al. (1993) have reported the possible effect of acupuncture in maintaining cardiovascular homeostasis in healthy people. Li has reported that the salutary effect of electroacupuncture (EA) is related to the diminished cardiac oxygen demand during myocardial ischemia (Li et al., 1998). Previous studies in our lab have shown enhancing effects on the cardiac function of rats with acute myocardial ischemia (Xia et al., 2008). Xia has proved the curative effect of acupuncture on rats with acute myocardial ischemia through decreased sympathetic outflow and improvement of cardiac functions (Xia et al., 2008). However, few experiments have been conducted to

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investigate the effect of acupuncture on cardiac function in surgical trauma-stressed animals.

The effects of acupuncture on cardiovascular diseases are related to the modulation of abnormal function of the autonomic nervous system (Li and Yao, 1992). The hypothalamic paraventricular nucleus (PVN) and the rostral ventrolateral medulla (RVLM) are considered the key areas modulating the function of the autonomic nervous system (Campos and McAllen, 1997; Swanson and Sawchenko, 1983). Sympathetic nerve activity can be regulated by neurons in the RVLM and the PVN. Neurons in the RVLM continuously transmit sympathetic nerve impulses to the heart and blood vessels to increase their activity. The effects of RVLM neurons on the heart include increasing heart rate (HR), conduction, and contractility, whereas the effects on vessels are predominantly vasoconstrictive (Despopoulos, 2003). Interconnections exist between the PVN and the RVLM (Swanson and Sawchenko, 1980, 1983). The PVN is an integrating center for the regulation of neuroendocrine, cardiovascular, and other physiological functions (Sonner and Stern, 2007; Zahner et al., 2007). It comprises two types of neurons: magnocellular and parvocellular neurons, which are functionally discrete. The parvocellular PVN contains neurons that send descending projections to the RVLM and the intermediolateral cell column of the thoracolumbar spinal cord (IML). It is reported that about 30% of spinally projecting neurons in the PVN also send collaterals to the RVLM. Thus, the neurons in the PVN can regulate sympathetic outflow via the above three pathways either directly or indirectly (Badoer, 2001; Pyner and Coote, 2000).

The present study was carried out to investigate the effects of EA application on cardiac function in rats that have experienced thoracic surgery-induced stress and to determine whether the PVN and the RVLM participate in regulating the cardiac function when EA is applied to the surgical trauma-stressed rats. The expression of c-Fos is a marker of neuronal activation (Morgan and Curran, 1991); thus, the present study determined the expression of c-Fos in the PVN and the RVLM in different groups to explore the underlying neurobiological mechanisms of EA actions, using a morphological approach. The electrophysiological properties of PVN neurons were also observed to further determine the mechanisms of EA action via an in vitro functional approach.

2. Materials and methods

2.1. Animal preparation

Experiments were carried out on male Sprague-Dawley rats (Shanghai Laboratory Animal Center, Chinese Academy of Sciences, China) weighing 250–300 g. All experimental procedures conformed to both international and Fudan University guidelines on the ethical use of animals, and all efforts were made to minimize the number of animals used and their suffering.

The rats were anesthetized with 6% chloral hydrate (360 mg/kg body weight, intraperitoneally). The depth of anesthesia was monitored by probing the pedal withdrawal reflex, as recommended, throughout the experiment (Zuurbier et al., 2002). Supplemental chloral hydrate was administered to maintain an adequate depth of anesthesia as judged by the lack of a withdrawal response to fascia pinch. An arterial catheter was inserted into the left femoral artery for measurement of blood pressure (BP), and another catheter was inserted into the left ventricle of the heart through the right carotid artery for recording the left intraventricular pressure, using two separate pressure transducers. Then, the two catheters were connected to a bioelectric signal-processing system (Model SMUP-E, Department of Physiology and Pathophysiology, Shanghai Medical College of Fudan University), from which the data related to cardiac function were obtained and analyzed. The analyzed parameters of cardiac function included HR, mean arterial pressure (MAP), left ventricular end-diastolic pressure (LVEDP), maximal rates of increase ($+dP/dt_{max}$) and decrease ($-dP/dt_{max}$) in the left intraventricular pressure, and the total area of the cardiac force loop (CFL, usually expressed as L_0). The values of $+dP/dt_{max}$ and $-dP/dt_{max}$ were obtained from the time derivative of the left ventricular pressure in a cardiac cycle. The cardiac force loop (L_0) is the loop with the time derivative of the left ventricular pressure (dP/dt) vs. the left ventricular pressure on the abscissa. Trapezoidal integration was used to obtain the total area of the CFL (L_0), which can evaluate myocardial contractility (Grossman et al., 1971; Marble et al., 1981). During the experiment, the body temperature was measured using a rectal thermometer and maintained at $37.5 \pm 0.5\,^{\circ}C$ using a temperature controller (H-KWDY-III, Quanshui Experimental Instrument, China).

2.2. Surgical procedure

After animal preparation, the surgery-induced stress was inflicted as follows. The rats were intubated and ventilated with a ventilator (DHX-50, Chengdu Instrument Company, China). A 4-cm-long left anterior thoracotomy was then effected longitudinally along the third and the fourth ribs; afterward, the thoracic cavity was exposed for 60 min. After surgery, the wounds were sutured and the ventilator removed. During the experiment, the animals were maintained warm, and the arterial pH, p_{CO_2}, and p_{O_2} were monitored using a blood gas analyzer (Medica Easy Blood Gas Analyzer; Medica, Bedford, MA, USA) and maintained within normal limits (p_{CO_2}: 30–35 mmHg and p_{O_2}: >100 mmHg). The pH of arterial blood was maintained within the range of 7.35–7.45.

The arterial and left intraventricular pressures were recorded at the preoperative stage and at 60 min after the suturing of the wound.

2.3. EA application

Stainless steel needles were inserted into the bilateral "Neiguan" points (PC6), corresponding to that of humans, which are located 3 mm above the wrist between the ligaments of flexor carpi radialis and palmaris longus in rats (Li and Yao, 1992). The "Lieque (LU7)" acupoints, located above the radial styloid process, between the tendons of brachioradialis and abductor pollicis longus, corresponding to that of humans, were used as controls. Needle insertion and stimulation parameters were the same as for the Neiguan acupoints. The electric impulses were produced by a medical stimulator (G6805-2, Shanghai Medical Instruments Hi-Tech Co., Ltd., China) at a frequency of 5 Hz, with 0.5-ms duration and an intensity ($\leq$4 mA) just strong enough to elicit slight twitches of the forelimb. EA stimulation was applied to the bilateral Neiguan or Lieque points during the thoracotomy operation and lasted for 30 min.

2.4. Immunohistochemistry

Rats were anesthetized with 6% chloral hydrate (360 mg/kg body weight, intraperitoneally) and perfused through the ascending aorta with 200 ml heparinized saline, followed by 200 ml of freshly prepared 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) at 60 min after the thoracotomy operation. After perfusion, the brains were removed from the skulls and postfixed at 4 °C overnight. Tissues from unstressed and stressed animals were handled in an identical manner. The fixed brains were placed in a solution of 20% sucrose until the brain sunk to the bottom and then placed in a solution of 30% sucrose in 4% paraformaldehyde at 4 °C overnight. Coronal sections of the brain (30 μm) were obtained using a cryostat microtome (Leica CM 1900, Germany). Immunohistochemical visualization was conducted using a conventional avidin–biotin–peroxidase complex (ABC) procedure. All experiments were conducted at room temperature ($23 \pm 2\,^{\circ}C$), unless stated otherwise. Free-floating brain slices were washed in 0.01 M phosphate-buffered saline (PBS; pH 7.4) and incubated in 0.01 M PBS containing 0.3% Triton X-100 for 30 min. The slices were then rinsed sequentially in PBS (three times), in 0.3% hydrogen peroxidase/PBS for 10 min to inactivate endogenous peroxidase, and then again three times in PBS, after which the slices were blocked with 5% normal goat serum for 20 min. A commercially available antibody, directed at amino acid residues 4–17 in human c-Fos (rabbit anti-c-Fos AB5, Calbiochem, La Jolla, CA, USA), was used to stain one set of free-floating sections from each rat for the presence of Fos. The preparations were incubated at 37 °C for 2 h and then incubated at 4 °C overnight with the diluted primary antibody (1:5000). After rinsing with PBS, the sections were subjected to exposure to anti-rabbit IgG and subsequently to the ABC complex (Shanghai Shen-Hang Bio-Tech Co., Ltd., China). Then, 3,3′-diaminobenzidine (DAB, Shanghai Shen-Hang Bio-Tech Co., Ltd., China) was applied to visualize the immunostained sections. Immunostaining in the absence of primary or secondary antibody was assessed for background evaluation.

In addition, the sections were examined using light microscopy to identify c-Fos-positive cells in the RVLM and the PVN regions, which were identified based on the stereotaxic atlas of the rat brain, proposed by Paxinos and Watson (1997). The number of Fos-immunoreactive (Fos-IR) neurons was counted using the software Image Measurement Version 1.00 (Department of Physiology and Pathophysiology, Shanghai Medical College, Fudan University, China). Fos-IR neurons were counted in six sections per rat, separately for each side of the brain, for the various brain nuclei investigated. The average number of c-Fos-IR neurons per section was calculated in each rat for the brain nuclei mentioned above. This procedure was carried out in a strictly single-blind method.

2.5. In vitro electrophysiology

At 60 min after the operation, rats were decapitated and their brains rapidly removed and immersed in ice-cold, oxygenated (by 95% O_2 and 5% CO_2) artificial cerebrospinal fluid (ACSF), containing (in mM): 124 NaCl, 3 KCl, 2.4 $CaCl_2$, 26 $NaHCO_3$, 1.3 $MgSO_4$, 1.4 NaH_2PO_4 and 11 glucose (Tasker and Dudek, 1991). One