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Research report

The protective effects of electro-acupuncture in thoracic surgery on trauma stressed rats involve the rostral ventrolateral medulla and supraoptic nucleus

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ABSTRACT

The present study was designed to explore whether the rostral ventrolateral medulla (RVLM) and supraoptic nucleus (SON) were involved in the protective effects of electro-acupuncture (EA) in thoracic surgery on trauma-stressed rats. The rats were randomly divided into a non-stressed group (Control), surgical trauma-stressed group (Trauma), and Neiguan EA applied on the surgical trauma-stressed group (Trauma + EA-PC 6). RVLM neuron discharge was observed by using an *in vivo* electrophysiological method, and micro-dialysis combining high-performance liquid chromatography with fluorometric detection (HPLC-FD) was used to assess expression of amino acids in the RVLM. Immunohistochemical methods were used to assess c-Fos expression in SON neurons. The trauma of surgical stress was shown to dramatically increase the discharge frequency of RVLM neurons and promote the release of glutamate and taurine in the RVLM. The expression of c-Fos was also significantly increased in the SON of traumatized rats. EA application at Neiguan acupoints significantly suppressed trauma-induced increase of discharge frequency of the RVLM neurons, almost completely suppressed the trauma-induced increase of glutamate release but only very slightly reduced the trauma-enhanced taurine release, and inhibited the increase of c-Fos expression in these SON neurons of traumatized rats. These results indicate that Neiguan EA may improve cardiac function by modulating neurons in the RVLM and the SON in surgically traumatized rats. The taurine-mediated negative feedback may be involved in the protective effect of EA on cardiac function.

1. Introduction

Our previous study demonstrated that electro-acupuncture (EA) application at Neiguan (PC 6) acupoints can enhance cardiac function in thoracic surgery trauma–stressed rats. The protective effects of EA may be mediated by paraventricular nucleus (PVN) and rostral ventrolateral medulla (RVLM) neurons (Zhang et al., 2012). EA is a traditional method of Chinese medicine treatment, which may be a promising method of alleviating surgical trauma and helping patients with postoperative recovery. However, the related neurobiological mechanisms underlying EA action remain unclear yet, such as the neurotransmitters or modulators mechanisms of EA effects in the RVLM. It is also unclear whether the protective effects of EA on surgical trauma rats involve other central nuclei in autonomic nervous system.

It is well known the RVLM is a critical center regulating cardiovascular function (Jiang et al., 2011a; Jiang et al., 2011b; Sapru, 2002). The regulation involves a variety of neurotransmitters and receptors, including excitatory and inhibitory amino acids, nitric oxide (NO), opioid peptides, angiotensin II type 1 (AT1) receptors, and 5-hydroxytryptamine receptors in the RVLM (Dampney et al., 2002; Kishi, 2013; Ramage, 2001; Sved et al., 2002). All are also involved in the regulation of cardiovascular activity. Our previous studies showed that amino acid neurotransmitters (including excitatory and inhibitory amino acids) in the RVLM are essential for cardiovascular regulation. These neurotransmitters may be the mediators of other transmitters or modulators playing a role (Hu et al., 2002; Wang et al., 2003; Zhu et al., 1998). In the RVLM, many neurotransmitters, such as GABA, opioids, serotonin, nociceptin, endocannabinoids, are also modulated by EA

Abbreviations: AT1, angiotensin II type 1; DA, dopamine; EA, electro-acupuncture; HPLC-FD, high-performance liquid chromatography with fluorometric detection; Glu, glutamate; NE, norepinephrine; NO, nitric oxide; OT, oxytocin; PVN, paraventricular nucleus; RVLM, rostral ventrolateral medulla; SON, supraoptic nucleus; Tau, taurine; VP, vasopressin

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(Moazzami et al., 1985; Tjen et al., 2007).

The supraoptic nucleus (SON) is another key area in the hypothalamus which participates in regulating cardiovascular function (Cunningham et al., 2002). The SON primarily comprises two types of magnocellular neurosecretory cells, oxytocin (OT) and vasopressin (VP) neurons, as well as other interneuron. The SON is also an important center for participation in the stress response (Briskiet al., 2001; Yang et al. 2014). C-Fos is a molecular marker neuron activation (Kovacs, 1998). Fos-immunopositive neurons in the SON increase following immobilization stress and restraint water-immersion stress (Briski et al., 2001; Sun et al. 2016). Lee et al. reported that immobilization stress-induced Fos-like immunoreactivity of the parvocellular SON was significantly attenuated by EA at HT3-PC6 (Lee et al., 2004). Little is yet known about the effect of surgical trauma stress on the SON. In particular it is not known whether protection of cardiac function by applying EA at PC 6 acupoints involves the SON, and whether the SON regulates cardiovascular function by having an effect on the RVLM.

The present study was designed to observe electrical activity and release of amino acid neurotransmitters in RVLM neurons using *in vivo* extracellular recording methods and micro-dialysis with high performance liquid chromatography-fluorescence detection (HPLC-FD) methods to evaluate the RVLM mechanisms underlying the protective effects of EA on cardiac function in trauma stressed rats. C-Fos expression in the different groups was observed using immunohistochemical methods in order to analyze whether the SON participates in the protective effects of EA on cardiac function in surgical trauma stressed rats.

2. Materials and methods

2.1. Animal preparation

Male Sprague-Dawley rats, weighing 250–300 g, were purchased from Shanghai Laboratory Animal Center (Shanghai, China) and Qinglongshan Animal Breeding Grounds (Nanjing, China). All experimental procedures were approved by Experimental Animal Ethics Committee of Fudan University.

Rats were randomly divided into a non-stressed group (Control), surgical trauma stressed group (Trauma), and a Neiguan EA applied on surgical trauma stressed group (Trauma + EA-PC 6). Rats were anesthetized with a 6 ml/kg intraperitoneal injection of composite anesthetic agent (14 g urethane, 0.7 g chloralose and 0.7 g borax per 100 ml normal saline). During the experiment, the pedal withdrawal reflex was probed to assess the effect of anesthesia (Alves et al. 2010). Adequate anesthesia could be maintained by supplemental administration of the mixed anesthetic. The appropriate level of anesthetic was judged by the lack of a withdrawal response to fascia pinch. As described in the previous article (Zhang et al., 2012), to produce a stress response, the rats were subjected to a 4-cm-long left anterior thoracotomy and the thoracic cavity was exposed for 60 min. During the procedure, the rats were always intubated and ventilated with a ventilator (DHX-50, Chengdu Instrument Company, China). Until 60 min later, the incision was sutured and the ventilator was removed. In the Trauma + EA-PC 6 group, EA was performed at bilateral “Neiguan” points during the thoracotomy operation. Electrical stimulation was applied via the needles using a medical stimulator (0.5 ms, 5 Hz, 30 min, G6805-2, China). The stimulation intensity ($\leq$4 mA) was just strong enough to cause slight twitches of the forelimb.

2.2. In vivo electrophysiology

Rat anesthesia was the same as mentioned above. At 60 min after thoracotomy trauma, the rat was placed on the constant temperature pad (ALC–HTP101N, Shanghai Alcott Biotech Co., Ltd., China) to maintain body temperature at 37.5 ± 0.5 °C. The head was fixed on the stereotactic apparatus (Model 68002, Shenzhen Ruiwo De Life Technology Co., Ltd., China). The skull was exposed and leveled between bregma and lambda. The RVLM was located per the atlas of Paxinos and Watson (Paxinos and Watson, 2004) at the following stereotaxic coordinates (in mm): −12.00 to −12.36 mm from the bregma, 1.9–2.4 mm lateral to midline, and 10.2–10.7 mm below the skull surface. A burr hole was drilled over the RVLM and the meninges was opened. The recording microelectrodes were pulled from glass capillaries and filled with a 2% solution of Chicago sky blue in 0.5% sodium acetate. The electrical signals of the RVLM neurons were recorded at a resistance of 5–15 MΩ. Signals were input to a PowerLab data acquisition and analysis system (AD Instruments Inc., Sydney, Australia) via a microelectrode AC amplifier (Model 1800, A-M Systems, USA), band-pass filtered between 0.1 and 5.0 kHz. Labchart software (AD Instruments Inc., Sydney, Australia) was used to analyze the recorded data. The threshold signal amplitude (85 μV) was set for the frequency measurements for all animals.

At the end of the experiment, the recording sites of the RVLM were marked by electrophoretic application of a 2% solution of Chicago sky blue (10–100 μA of negative currents for 20 min or 10 min, then 10–100 μA of positive currents for 16 min or 8 min). The brains were removed and fixed in 4% formaldehyde solution for 4–7 days. Coronal slices were prepared and the position of the Chicago sky blue marking observed. The marking was compared with the atlas of Paxinos and Watson to identify the recording sites.

2.3. Micro-dialysis and high-performance liquid chromatography with fluorometric detection (HPLC-FD)

Rat anesthesia was done as mentioned above. Tracheal intubation was performed immediately after the operation, and the rats were then fixed in the prone position on the stereotaxic apparatus and the head leaned forward 45°. The fourth ventricle was exposed by carefully removing the occipital bone, and the floor of the fourth ventricle was kept horizontal. A micro-dialysis probe (effective dialysis membrane length 2 mm, outside diameter 0.2 mm, MAB, Sweden) was vertically inserted into the RVLM (1.8–2.0 mm ahead of the obex, 1.6–2.0 mm right to the midline, and 3.0–3.4 mm depth from the medullary dorsal surface) through the fixed catheter. The inlet end was connected to the micro-dialysis syringe pump (Bioanalytical Systems, MD-0100, USA) through a plastic catheter and the outlet was connected to a 0.2 ml centrifuge tube through a catheter to collect dialysate for high performance liquid phase determination. The perfusate solution for micro-dialysis comprised an artificial cerebrospinal fluid (ACSF) (pH7.4, composition in mM: NaCl 130, KCl 2.99, $CaCl_2$ 0.98, $MgCl_2{\cdot}6H_2O$ 0.80, $NaHCO_3$ 25, $Na_2HPO_4{\cdot}12H_2O$ 0.039, $NaH_2PO_4{\cdot}2H_2O$ 0.46), which was perfused at rate of 2 μl/min. One dialysate sample (20 μl) was collected at post-operative 60 min and 120 min respectively.

The amino acids in the samples were assessed using a HPLC system (Agilent 1100 Series, Agilent Technologies Inc., USA). The chromatographic column was a reversed-phase column (C18, Ultrasphere ODS, 4.6 mm × 25 cm, particles 5 μm). The amino acids were quantified with an o-phthaldialdehyde derivative and fluorescence detection detected at a 330 nm excitation wavelength and 450 nm emission wavelength. The injection volume was 20 μl. The mobile phase consisted of 0.1 M phosphate buffer (70%, pH 6.0), methanol (28.4%), tetrahydrofuran (1.6%), and the flow rate was 1 ml/min. The column temperature was set at 25 °C.

To assist in locating sites of micro-dialysis, a 0.1 μl of 2% Chicago sky blue solution was injected at the micro-dialysis site at the end of each experiment. Brains were then removed and fixed in 4% paraformaldehyde solution for 4–7days. Brains were coronally sectioned using a sharp blade to visualise the dye spot and verify its placement at the RVLM using the atlas of Paxinos and Watson.

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