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Acupuncture pretreatment protects heart from injury in rats with myocardial ischemia and reperfusion via inhibition of the β_1-adrenoceptor signaling pathway

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Abstract

Our previous study showed that a cardioprotective effect was produced by pretreatment with acupuncture at bilateral Neiguan acupoints (PC6) and the effect of EA was diminished by propranolol, a nonspecific antagonist of β-adrenoceptors (β-ARs) which are the most powerful cardiac receptors, indicating an involvement of β-ARs. The present study explored further the signaling mechanism underlying the cardioprotective effect of acupuncture pretreatment in rats subjected to myocardial ischemia and reperfusion (MIR). Myocardial ischemia was achieved by ligating the left anterior descending coronary artery and reperfusion by releasing the ligation. Adult rats were divided into three groups, namely, a normal control (NC) group, a group subjected to ischemia and reperfusion (IR) only, and a group given electro-acupuncture (EA) before IR. For EA, bilateral Neiguan points (PC6) of the rats were stimulated for 30 min once a day for 3 consecutive days. The ST segment of ECG, the ratio of infarct size over risk zone, and the contents of β_1-adrenoceptor (β_1-AR), Gsα protein and cAMP in ischemic myocardium were compared among the three groups. IR increased the elevation of ECG ST segment, myocardial infarct size, contents of β_1-AR, Gsα protein and cAMP. These effects were attenuated by EA pretreatment at bilateral Neiguan acupoints. In conclusion, the present results indicate that EA produces cardioprotective effect against IR which may be mediated via the β_1-AR–Gs-protein–cAMP pathway.

Keywords: Electro-acupuncture; Pretreatment; Ischemic injury; β_1-adrenoceptor; Signal transduction

Introduction

Myocardial ischemic preconditioning (cyclic brief ischemia) is known to effectively protect the heart from injury induced by subsequent sustained myocardial ischemia and reperfusion (MIR) (Murry et al., 1986). Our preliminary study showed that repetitive pretreatment with electro-acupuncture (EA) at bilateral Neiguan acupoints also produced protective and anti-arrhythmic effects in rats subjected to MIR by mimicking the ischemic preconditioning (Gao et al., 2006). These effects of EA pretreatment were attenuated by propranolol, a β-adrenoceptor (β-AR) antagonist, indicating an involvement of cardiac β-ARs. It is well known that β_1-adrenoceptor (β_1-AR) plays a crucial role in both the regulation of cardiac physiological activity and the mediation of myocardial injury induced by ischemia and reperfusion. In addition, repetitive stimulation of either the β_1-AR or the components of its signaling pathway before MIR was shown to attenuate the myocardial injury induced by the prolonged ischemia and reperfusion (Nasa et al., 1997; Frances et al., 2003; Cain et al., 1998), in a manner similar to the cardioprotection produced by ischemic preconditioning. Thus, repetitive pretreatment with acupuncture may produce a cardioprotective effect via an influence on β_1-AR and/or its signaling pathway.

According to the theory of Traditional Chinese Medicine, Neiguan is an acupoint of the pericardial meridian and has been commonly used for treating cardiovascular diseases in China and other countries (Ho et al., 1999; Richter et al., 1991; Middlekauff, 2004; Zhang and Xu, 2004). The results of our

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previous study showed that pretreatment with EA at bilateral Neiguan acupoints protects the heart from injury by ischemia and reperfusion, while pretreatment with EA at non-acupoints does not produce significant myocardial protection (Gao et al., 2006).

Therefore, the aim of the present study was to explore whether the β_1-AR and its signaling pathway are involved in the mediation of the cardioprotection afforded by EA pretreatment at bilateral Neiguan acupoints (PC6) by measuring the contents of myocardial β_1-AR, Gs protein and cAMP together with ST segment of ECG and infarct size in rats subjected to MIR. The results showed that EA significantly attenuates MIR-induced cardiac injury accompanied by reductions in β_1-AR, Gs protein and cAMP. The finding suggests that the effect of EA pretreatment may be mediated via the β_1-AR, Gs-protein and cAMP pathway.

Materials and methods

Animal model of myocardial ischemia and reperfusion

The present study was approved by the Committee in the Use of Live Animals in Research of China Academy of Chinese Medical Sciences. Male Wistar rats weighing 266–320 g were divided into three groups, namely normal control (NC) group, ischemia–reperfusion (IR) group and electro-acupuncture (EA) group. Before the experiments the animals in EA group were pretreated with EA applied at bilateral Neiguan acupoints (PC6, according to the textbook of experimental acupuncture, Neiguan acupoint is located on forelimbs) for 30 min once a day for three consecutive days. For the acupuncture manipulation, two needles, 2–3 mm apart from each other, were inserted through the skin to a depth of about 2 mm at each Neiguan acupoint. The two needles puncturing at each Neiguan acupoint were connected to positive and negative poles of acupuncture apparatus. The stimulatory intensity and frequency of EA were 5 mA and 20 Hz respectively. On the day of the experiment the chests of all the rats were opened surgically under anesthesia with urethane. After a recovery of 20 min following the surgical operation, the left descending branch of coronary artery in the rat was ligated with silk snare for 30 min followed by a 15-min reperfusion among the IR and EA groups. The right femoral artery of the rats was cannulated with polyethylene tubing connected to a pressure transducer (MP-6S, Nihon Kohden) and the arterial blood pressure was monitored on data acquisition system (PowerLab, ADInstruments) throughout the experiment. The same operation without the ligation of coronary artery was performed in the rats of NC group.

ECG ST-segment evaluation

Electrocardiograms (ECG) were recorded continuously with standard lead II before, during and after MIR in all the animals by use of a computerized PowerLab system (ADInstruments, Australia). ST segment of ECG elevated over 100 μV from baseline was recruited as an index of ischemia. The amount of elevated voltage in μV in the different groups was compared statistically.

Determination of ischemic risk zone and infarct size

When the experiment in vivo was finished the heart was cut off and perfused with Krebs solution in a retrograde manner in the Langendorff perfusion system (Radnoti, USA) as described previously (Yu et al., 2001a). Evans blue at the concentration of 0.25% was infused into the heart to determine the myocardial risk zone. The heart was then frozen and cut into 2-mm thick slices. The parts of right ventricle and connective tissue were removed. The slices were incubated in 1% 2,3,5-triphenyltetrazolium chloride (TTC) buffer at pH 7.4 for 15 min at 37 °C and were then immersed in 10% formalin overnight. The infarct (TTC negative) and risk zone (TTC stained) were measured by a computerized planimetric technique (Minichromax; Biolab) (Wang et al., 2001). In the present study the infarct severity was presented as the ratio of infarct size/risk zone.

Ischemic myocardium collection

After the experiment in vivo the heart was isolated and perfused with Krebs solution in a retrograde manner in the Langendorff perfusion system as described above. Evans blue at the concentration of 0.25% was infused into the heart to determine the myocardial risk zone (Evans blue negative). The ischemic myocardium was cut off and frozen rapidly in liquid nitrogen after removal of atria and right ventricle. The frozen left ventricle was weighed and stored at −70 °C.

Measurement of cardiac β_1-AR

On the day of preparation of ventricular sarcolemma, the stored ventricle was homogenized in hypotonic membrane buffer containing 1 mM 1,10-phenanthroline, 1 mM iodoacetamide, 1 mM pepstatin A, 0.4 mM phenylmethylsulfonyl fluoride (PMSF) with ultrasound homogenizer (Sonics and Material Inc, Jencons Ltd. Germany) in three bursts (7 s each) at an interval of 30 s. The homogenate was then centrifuged at 100 ×*g* for 5 min. The supernatant was further centrifuged at 15,000 ×*g* for 25 min with high speed freezing centrifuge (Hettich, Germany). The pellet was washed once with the membrane buffer, recentrifuged, and finally suspended in the membrane buffer and stored at −70 °C for further experiments.

A modified Western blotting technique was used to determine the content of cardiac β_1-AR (Podlowski et al., 1998). In brief, sodium dodecyl sulfate gel electrophoresis of polypeptides was performed on 10% polyacrylamide gels with electrophoresis apparatus (Bio-Rad Laboratories, USA). The samples, containing 80 μg of protein, were added with the same amount of the sample loading buffer (reducing buffer) and then heated at 100 °C with boiling water for 5 min. The solution was added into a single lane of the gel. After the gel was run, the polypeptides were transferred electrophoretically onto a nitrocellulose membrane. The membrane was then washed with a Tris buffer solution (TBS) and incubated with TBS containing 5% nonfat dry milk (blocking buffer) for blocking the nonspecific protein binding sites on nitrocellulose. An antibody to β_1-AR was added at 1:1000 dilution in the blocking buffer