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Non-excitatory electrical stimulation attenuates myocardial infarction via homeostasis of calcitonin gene-related peptide in myocardium

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ABSTRACT

Electrical stimulation has been shown protection of brain, retina, optic nerves and pancreatic β-cells but the effect on cardio-protection is still unknown. Calcitonin gene-related peptide (CGRP) participates in the pathology of injury and protection of myocardium but whether or not electrical stimulation modulates endogenous CGRP is not clear. Male Sprague-Dawley rats were divided into 4 groups: (1) control group, without any treatment. (2) I/R group, animals were subjected to 30 min of myocardial ischemia followed by 60 min reperfusion. (3) NES + I/R group, non-excitatory electrical stimulation (NES) was commenced from 15 min before coronary artery occlusion till the end of reperfusion. (4) I/R + $CGRP_{8-37}$ group, animals were given with $CGRP_{8-37}$ (an antagonist of CGRP receptor, 10^{-7} mol/L, 0.3 ml, *i.v.*) at 5 min before reperfusion without any electrical stimulation. The hemodynamics and electrocardiogram were monitored and recorded. Infarct size and troponin I were examined and CGRP expression in the myocardium and serum was analyzed. It was found that the infarct size and TnI were significantly reduced in NES + I/R group, by 45% and 58% respectively, accompanied by an obvious fall back of CGRP in myocardium, compared to I/R group (all $p < 0.05$). Treatment with $CGRP_{8-37}$ resulted in the same protection on myocardium as NES did. No significant difference in hemodynamics or ventricular tachycardia was detected among the groups (all $p > 0.05$). It can be concluded that NES reduced the infarction size after acute myocardial ischemia and reperfusion, for which the underlying mechanism may be associated with modulation of endogenous CGRP in myocardium.

Introduction

Reperfusion of myocardium is an effective therapy for treatment of patients suffering from ischemic heart disease and also an inevitable course for some cardiac surgeries, such as cardiopulmonary bypass, which potentially exacerbates myocardial injury after re-opening of coronary circulation following myocardial ischemia. The reperfusion injury is characterized by exaggerated myocardial necrosis, apoptosis, dysfunction and lethal arrhythmias [1–3].

Progresses have been made in management of the myocardial injury, especially by demonstration of the cardio-protective effects of pre- and post-conditioning the heart with transient myocardial ischemia at the beginning of myocardial ischemia or reperfusion, respectively, before prolonged reperfusion of ischemic myocardium. As a multifunctional sensory neuropeptide, calcitonin gene-related peptide (CGRP) known as a vasodilator, has been showing participating in cardio-protection [4–6]. Efforts have been made using ischemic [4,5], pharmacological [7] and genetic [8,9] approaches for induction of cardio-protection via modulation of CGRP. Lines of evidence indicate that electrical stimulation on cardiac nerves may regulate release of neurotransmitters [10,11], shedding light on a potential path for the limitation of myocardial injury by electrical stimulation via modulation of endogenous CGRP.

However it was reported that electrical stimulation resulted in adverse effects when the intensity of stimulation was high enough to pace the heart, in vivo [12], or in vitro [13,14]. On the contrary,

Abbreviations: I/R, ischemia and reperfusion; CGRP, calcitonin gene-related peptide; CCM, cardiac contractility modulation; NES, non-excitatory electrical stimulation; TTC, 2,3,5-triphenyltetrazolium chloride; ECG, electrocardiogram; VPBs, ventricular premature beats; VT, ventricular tachycardia; VF, ventricular fibrillation; AAR, area at risk; IS, infarct size; LA, left ventricular area; ELISA, enzyme-linked immunoassay.

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Table 1
Protocol of the study.

Experiments	Groups and numbers of animals (n) involved			
	Control ($n=6$)	I/R ($n=12$)	NES + I/R ($n=12$)	I/R + $CGRP_{8-37}$ ($n=6$)
TTC	–	$n=6$	$n=6$	$n=6$
ELISA	$n=6$	$n=6$	$n=6$	–
Hemodynamics	–	$n=12$	$n=12$	$n=6$
Arrhythmias	–	$n=12$	$n=12$	$n=6$

I/R, ischemia and reperfusion; NES, non-excitatory electrical stimulation; TTC, staining with 2,3,5-triphenyltetrazolium chloride; ELISA, enzyme linked immunosorbent assay.

beneficial effects of non-excitatory electrical stimulation (NES) on cardiac performance [15] and survival [16] were reported. But, it is still unclear whether or not the non-excitatory electrical stimulation produces cardio-protection limiting the reperfusion injury.

Based on the findings, we designed this study to evaluate the potential effect of non-excitatory electrical stimulation on myocardial injury induced by acute myocardial ischemia and reperfusion and the role of CGRP in the intervention, if the protective action of NES was established.

Materials and methods

Ethics statement

The study was conformed to the EU Directive 2010/63/EU for animal experiments and approved by the Institutional Animal Care and Use Committee of Shanxi Medical University. All efforts were made to minimize the number of animals used and their suffering.

Experimental protocol

Male Sprague-Dawley rats of 10–12 weeks old (350 ± 20 g) were randomly assigned to 4 groups (Table 1). The severity of myocardial injury was adjudged by analysis of infarction size and concentration of serum troponin I (TnI). The amount of CGRP in the myocardium at risk of ischemia and serum was measured. Animals in I/R group were subjected to 30 min of myocardial ischemia followed by 60 min of reperfusion. The NES was started at 15 min prior to the beginning of occlusion of coronary artery until the end of myocardial reperfusion, for NES + I/R group. $CGRP_{8-37}$, a selective antagonist of CGRP receptor, (Sigma–Aldrich, St. Louis, MO, USA) was injected through caudal vein at 10^{-7} mol/L (in 0.3 ml), at 5 min before start of reperfusion, in I/R + $CGRP_{8-37}$ group (Fig. 1). The samples of normal group were collected under anesthesia without I/R or NES as controls.

Model of myocardial ischemia/reperfusion

Rats were anesthetized with urethane (20%, 1.5 g/kg) via intraperitoneal injection. The animal was ventilated with room air with tidal volume of 20 ml/kg at a respiratory rate of 80 breathes/min, after a tracheotomy and an intubation were carried out. The right common carotid artery was catheterized for continuous hemodynamic monitoring, and body-surface electrocardiogram (ECG, lead II, RM6240BD, Chengdu, China) was obtained. A thoracotomy was performed at the left fourth intercostal space. The left anterior descending (LAD) branch of coronary artery was identified and ligated reversibly used a 5-0 silk suture through a polyethylene snare. Then the wound of the chest was covered by gauze without closure while the animal was mechanically ventilated throughout the experiment. Ischemia was confirmed by a discoloration of the myocardium and an elevation of ST segment (>0.1 mV) in ECG. Reperfusion was indicated by rapid disappearance of cyanosis and a fall back of the ST segment (>1/2, Fig. 2).

Electrical stimulation

Electrical stimulation was performed using two electrodes with the cathode placed in the muscles of chest wall and the anode pricked and fixed in the right ventricular wall. The protocol of stimulus application (RM6240BD, Chengdu, China) was a consecutive biphasic square-wave pulse (5 ms), setting the intensity of maximal sub-threshold voltage, not initiating any ventricular beat. And the frequency was confined to 65–70% of the spontaneous heart rate of the test animals. Recordings of ECG and carotid arterial pulses during NES confirmed a non-pacing property of the delivered electrical stimuli (Fig. 3).

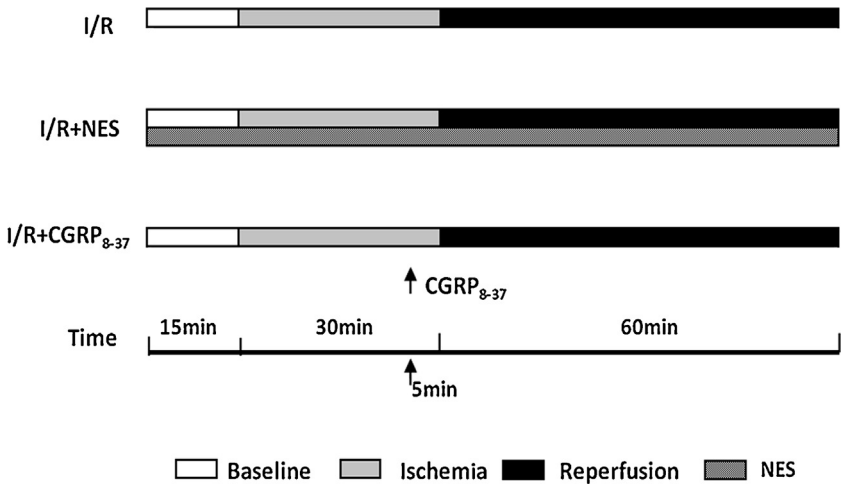

Fig. 1. Experimental protocol. I/R, ischemia and reperfusion group; I/R + NES group, treated with non-excitatory electrical stimulation or $CGRP_{8-37}$ (I/R + $CGRP_{8-37}$ group). The time of administration of $CGRP_{8-37}$ was indicated by the arrows.