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Vasopressin attenuates ischemia–reperfusion injury via reduction of oxidative stress and inhibition of mitochondrial permeability transition pore opening in rat hearts

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ABSTRACT

Aim of this study was to investigate the involvement of the mitochondrial permeability transition pore (MPTP) and oxidative stress in the cardioprotective effect of vasopressin (AVP) on ischemia/reperfusion (I/R) injury. Anesthetized male wistar rats were subjected to regional 30 min ischemia and 120 min reperfusion and randomly divided into nine groups: (1) Control; saline was administered intravenously before ischemia, (2) vasopressin was administrated 10 min prior to ischemia, (3, 4) Atractyloside as MPTP opener, was injected 5 min prior to reperfusion without and with vasopressin, (5, 6) Cyclosporine A as a MPTP closer, was injected 5 min prior to reperfusion without and with vasopressin, (7) mitochondria were isolated from control group and $CaCl_2$ was added as MPTP opener and swelling inducer, (8) isolated mitochondria from Control hearts was incubated with Cyclosporine A before adding the $CaCl_2$ (9) $CaCl_2$ was added to isolated mitochondria from vasopressin group. Infusion of vasopressin decreased infarct size ($18.6 \pm 1.7\%$ vs. control group $37.6 \pm 2.4\%$), biochemical parameters [LDH (Lactate Dehydrogenase), CK-MB (Creatine Kinase-MB) and MDA (Malondialdehyde) plasma levels, PAB (Prooxidant–antioxidant balance)] compared to control group. Atactyloside suppressed the cardioprotective effect of vasopressin ($32.5 \pm 1.9\%$ vs. $18.6 \pm 1.7\%$) but administration of the Cyclosporine A without and with vasopressin significantly reduced infarct size to $17.7 \pm 4\%$ ($P < 0.001$) and $22.7 \pm 3\%$ ($P < 0.01$) respectively, vs. $37.6 \pm 2.4\%$ in control group. Also, vasopressin, similar to Cyclosporine A, led to decrease in $CaCl_2$-induced swelling. It seems that vasopressin through antioxidant effect and MPTP inhibition has created a cardioprotection against ischemia/reperfusion injuries.

1. Introduction

Cardiac ischemia occurs frequently in critically ill patients and is associated with increased mortality (Lim et al., 2010). Cardiac preconditioning represents the most potent and consistently reproducible method of rescuing heart tissue from undergoing irreversible ischemic damage. Unfortunately, the clinical value of ischemic preconditioning (IPC) is limited. None of the several identified pharmacologic agents that appear to limit reperfusion injury is available for clinical use (Yang et al., 2004). Vasopressin or arginine vasopressin (AVP) is essential for cardiovascular homeostasis (Holmes et al., 2001). Circulating levels of AVP, which are elevated during hypovolemia and during cardiac stress, mediate important physiological functions such as osmotic regulation, vasoconstriction, and release of adrenocorticotropic hormone (ACTH) (Zhu et al., 2013). In the previous study we have shown that AVP (0.03 μg/rat) has protective effects on ischemia/reperfusion (I/R) induced myocardial injury in rat heart (Nazari et al., 2011). Interestingly, other study confirmed the protective effects of AVP in low dose on myocardial injury of the ischemic reperfused heart (Pelletier et al., 2013; Zhu et al., 2013). Also vasopressin infusion is cardioprotective in models of myocardial ischemia (Okamura et al., 1999) and in patients with postcardiotomy shock (Dunser et al., 2002). Moreover, we reported that AVP (0.03 μg/rat) provides cardioprotection against heart I/R injury by its anti-oxidant action (Nazari et al., 2011). However, the exact mechanisms of cardioprotection of AVP remain poorly understood.

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Mitochondria are the most important cellular sources of reactive oxygen species production and are particularly susceptible to oxidative stress (Sastre et al., 2003). Indeed, conditions associated with post-ischemic reperfusion, such as reactive oxygen species accumulation, pH normalization and increases in [Ca^{2+}], create an ideal scenario for mitochondrial permeability transition pore (MPTP) opening (Zoratti and Szabo, 1995). Opening of the MPTP may result in mitochondrial swelling, collapse of mitochondrial membrane potential, uncoupling of mitochondrial oxidative phosphorylation and cytochrome C release, leading to both necrosis and apoptosis. It was demonstrated that preventing MPTP opening may be protective in isolated cardiomyocytes (Nazareth et al., 1991) and perfused hearts (Griffiths and Halestrap, 1993). In addition, modulation of MPTP opening has been observed in cardioprotection by both preconditioning (Xu et al., 2001) and post-conditioning (Argaud et al., 2005) of the heart. Inhibition of MPTP opening also exerts cardioprotection against I/R injury in mice induced in vivo (Wang et al., 2005).

The purpose of the present study was to determine whether AVP protects the heart at reperfusion through a mitochondrial pathway, specifically via inhibition of MPTP opening.

2. Materials and methods

Male Wistar rats (Weighing 280–310 gr) housed under standardized conditions 12-h light/dark cycle, 20–22 °C ambient temperature and 40–50% humidity with free access to fed standard rat chow and tap water. All animal care and experiments were conducted in accordance with the institutional guidelines of Tehran University of Medical Sciences (Tehran, Iran).

2.1. Surgical preparation

Anesthesia was achieved by administration of sodium thiopental (60 mg/kg, i.p.). Body temperature was maintained at 37 ± 1 °C. After a tracheotomy, all rats were ventilated with air and oxygen mixture by Parvalux rodent respirator (15 ml/kg stroke volume and 60–70 Breaths/min). The right carotid artery was dissected and a heparinized saline (100 U/ml) filled polyethylene-tubing catheter (PE-50) was inserted into the artery for blood sampling and hemodynamic monitoring. The femoral vein was cannulated to inject Evans blue and drugs. Lead-II electrocardiogram (ECG) and arterial hemodynamic parameters were continuously monitored and recorded throughout the experiment, using a computerized data acquisition system (ML750 PowerLab/4sp, AD Instruments). 10 min prior to the end of reperfusion period, the carotidal catheter was advanced to the Left Ventricle (LV) to record the functional parameters of LV (Smith et al., 1979).

Rats were given heparin (200 IU/kg, i.v.), and then the chest was opened by a left thoracotomy in the fourth rib to expose the heart. The pericardium was incised and a 6–0 silk suture was placed around the left anterior descending coronary artery (LAD) close to its origin. Both ends of the suture were passed through coronary ligator. Heart rate and blood pressure were allowed to stabilize for 15 min before the intervention protocols. Applying tension to the suture by ligator caused regional ischemia, and reperfusion was achieved by releasing the tension on the ligature. Ischemia was confirmed by ST elevation in ECG, or cardiac cyanosis subsequent decrease in blood pressure, and reperfusion was confirmed by epicardial hyperemia.

2.2. Experimental protocol

After a stabilization period following the surgical preparation, basal hemodynamic parameters were measured for 15 min before drug administration and the heart of all animals was subjected to 30 min ischemia and 120 min reperfusion (Fig. 1). Rats were randomly divided into nine groups: (1) Control; saline was administered intravenously before ischemia, (2) AVP0.03; vasopressin 0.03 μg/rat was infused within 10 min prior to ischemia ($n=13$), (3, 4) Atr and AVP+Atr; Atractyloside (5 mg/kg, i.v.), as a MPTP opener, was injected 5 min prior to reperfusion without and with the effective dose of AVP 0.03 μg/rat into two different groups ($n=13$), (5, 6) CsA and AVP+CsA Cyclosporine A (CsA, 5 mg/kg, i.v.), as a MPTP closer, was injected 5 min prior to reperfusion without and with the effective dose of AVP (0.03 μg/rat) into two different groups ($n=13$). (7) Control–$CaCl_2$; mitochondria were isolated from control group and 200 μmol/l $CaCl_2$ was added to induce MPTP opening and swelling ($n=4$), (8) CsA–$CaCl_2$; an aliquot of mitochondria from Control hearts was incubated with 1 μmol/l CsA for 2 min before the addition of 200 μmol/l $CaCl_2$ ($n=4$) (9) AVP–$CaCl_2$; mitochondria were isolated from AVP0.03 group and 200 μmol/l $CaCl_2$ was added to examine mitochondrial swelling ($n=4$).

2.3. Hemodynamic functions

Arterial blood pressure and heart rate (HR) were continuously monitored and recorded throughout the experiment. Left ventricular hemodynamic parameters such as Left ventricular End-Diastolic Pressure (LVEDP), left ventricular developed pressure [LVDP=LVSP (Left ventricular systolic pressure)−LVEDP], maximum rise and fall of LV pressures ($+dp/dt$ and $-dp/dt$ respectively) and RPP (Rate pressure product=LVDP × HR) were recorded at 10 min of end reperfusion.

2.4. Cardiac area at risk and infarct size determination

At the end of reperfusion, the coronary artery was reoccluded and 2 ml of Evans blue (2%) was injected intravenously to the femoral vein. Then, the heart was excised, cut into 2 mm slices. All slices were incubated with a 1% 2,3,5-triphenyltetrazolium chloride (TTC, in 0.1 M phosphate buffer, pH 7.4) stain for 15 min at 37 °C, to visualize the infarct area. Then they were fixed in 10% formalin to enhance the contrast of the Evans blue and TTC staining. Both surfaces of each section were scanned using Photoshop program (Adobe Systems, version 7.0). Total area at risk was expressed as a percentage of the left ventricles (AAR/LV). Infarct size was expressed as a percentage of the area at risk (IS/AAR).

2.5. Biochemical analysis

Blood samples were collected at the end of reperfusion for measurement of the cardiac enzymes, including creatine kinase-MB (CK-MB), lactate dehydrogenase (LDH) and Malondialdehyde (MDA). The heparinized samples were centrifuged at 5000g, for 15 min, and the plasma was removed and stored at −70 °C until the time they were assayed. The activity of CK-MB and LDH were analyzed using commercial kits (Pars Azmoon, Iran) by employing an autoanalyzer (Roche Hitachi Modular DP Systems, Mannheim, Germany). MDA content of samples was determined spectrophotometrically using a modification of the assay described by Schuh et al. (1978).

2.6. Prooxidant–antioxidant balance (PAB) assay

A modified PAB was applied based on a previously described method (Alamdari et al., 2007). The standard solutions were prepared by mixing varying proportions (0–100%) of 250 μM hydrogen peroxide with 3 mM uric acid (in 10 mM NaOH). 60 mg TMB (3,3′,5,5′-Tetramethylbenzidine) powder was dissolved in 10 ml DMSO; for preparation of TMB cation, 400 μl of TMB/DMSO was added in 20 ml of acetate buffer [0.05 M buffer, pH 4.5], and then 70 μl of fresh chloramine T (100 mM) solution