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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 (18.671.7% vs. control group 37.672.4%), 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 (32.571.9% vs. 18.671.7%) but administration of the Cyclosporine A without and with vasopressin significantly reduced infarct size to 17.774% ($P<0.001$) and 22.773% ($P<0.01$) respectively, vs. 37.672.4% 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.

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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

was added into this 20 ml, mixed well, incubated for 2 h at room temperature in a dark place; 25 U of peroxidase enzyme solution was added into 20 ml TMB cation, dispensed in 1 ml and put at −20 °C; in order to prepare the TMB solution 200 μl of TMB/DMSO was added into 10 ml of acetate buffer [0.05 M buffer, pH 5.8]; the working solution was prepared by mixing 1 ml TMB cation with 10 ml of TMB solution, incubated for 2 min at room temperature in a dark place and immediately used. Ten microliters of each sample, standard or blank (distilled water) were mixed with 200 μl of working solution, in each well of a 96 well plate, which was then incubated in a dark place at 37 °C for 12 min; at the end of the incubation time, 100 μl of 2 N HCl was added to each well; and measured in an ELISA reader at 450 nm with a reference wavelength of 620 or 570 nm. A standard curve was provided from the values relative to the standard samples. The values of the PAB are expressed in arbitrary HK units, which are the percentage of hydrogen peroxide in the standard solution. The values of the unknown samples were then calculated based on the values obtained from the above standard curve.

2.7. Preparation of mitochondria

Mitochondria were isolated from male rats by differential centrifugation with some modifications (Ghazi-Khansari and Mohammadi-Bardbori, 2007). In brief, after deep anesthesia, the

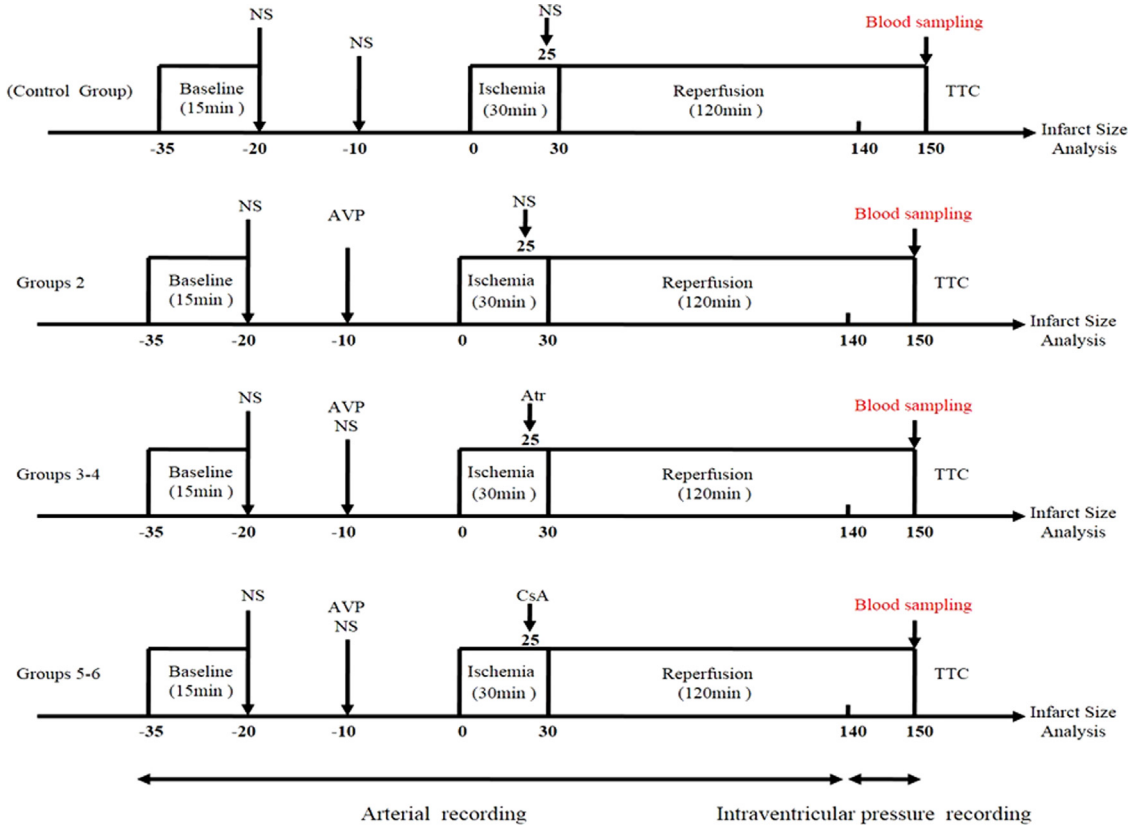

Fig. 1. Illustration of the experimental groups. Animals in control group were subjected to 30 min ischemia followed by 120 min reperfusion and saline was administered intravenously before ischemia, (group 2) AVP0.03; effective doses of vasopressin (0.03 μg/rat) was infused within 10 min prior to ischemia, (groups 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, (groups 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. NS, normal saline; TTC, triphenyltetrazolium chloride ($n=13$).

Table 1
Hemodynamic parameters.

Groups	Baseline		End of ischemia 30′		End of reperfusion 60′		End of reperfusion 110′	
	HR	SAP	HR	SAP	HR	SAP	HR	SAP
Control	308 ± 12	109 ± 5	290 ± 15	97 ± 5	269 ± 14[a]	92 ± 4[a]	233 ± 12[b]	76 ± 5[b]
AVP0.03	312 ± 18	110 ± 7	304 ± 14	97 ± 5	293 ± 15	87 ± 2[a]	293 ± 19[c]	85 ± 5[b]
Atr	376 ± 16	127 ± 6	354 ± 13	119 ± 3	351 ± 22[c]	100 ± 5[a]	357 ± 13[d]	95 ± 7[b]
AVP+Atr	334 ± 11	128 ± 5	338 ± 10	117 ± 4	340 ± 11[c]	104 ± 7[e]	331 ± 12[d]	84 ± 11[b]
CsA	334 ± 27	129 ± 5	316 ± 25	110 ± 6	322 ± 17	112 ± 5[c]	305 ± 14[f]	95 ± 5[a]
AVP+CsA	340 ± 10	124 ± 3	324 ± 18	108 ± 4[a]	324 ± 15	107 ± 5[a]	314 ± 15[f]	96 ± 5[b]

The values are mean ± S.E.M. HR, heart rate (beats/min); SAP, systolic arterial pressure (mmHg); AVP, arginine vasopressin; Atr, Atractyloside; CsA, Cyclosporine A.

[a] $P<0.01$ vs. Its baseline within group.
[b] $P<0.001$ vs. Its baseline within group.
[c] $P<0.05$ vs. Control.
[d] $P<0.001$ vs. Control.
[e] $P<0.05$ vs. Its baseline within group.
[f] $P<0.01$ vs. Control.

hearts immediately excised and AAR (area at risk) zone from LV was minced by small scissor in an ice cold manitol solution containing 0.255 M D-manitol, 74 mM sucrose and 0.2 mM ethylenediaminetetraacetic acid (EDTA) (pH=7.4). Then the minced tissue was gently homogenized in a glass homogenizer with a Teflon pestle and then centrifuged at 1500*g* for 10 min at 4 °C to remove nuclei, unbroken cells and other non-subcellular tissues. The supernatants were centrifuged at 10,000*g* for 10 min at 4 °C. The pellet was resuspended in 1 ml of respiration buffer (70 mM sucrose, 230 mM manitol, 3 mM HEPES, 2 mM Tris–phosphate, 5 mM succinate and 1 μM of rotenone) (pH=7.4) for the determination of mitochondrial swelling.

2.8. Protein concentration

Mitochondrial protein concentrations were determined using the method developed by Bradford (1976).

2.9. Mitochondrial swelling assays

Opening of the MPTP causes mitochondrial swelling and the change in mitochondrial volume due to colloidal osmotic effects of solute flux into and out of the mitochondrial matrix was measured by monitoring the absorbance at 520 nm (A_{520}) as described (Zhao et al., 2010). Briefly, isolated mitochondria (approximately 500 μg) in 1 ml of respiration buffer were pre-incubated at 30 °C for 10 min. After a 10 min equilibration period, 200 mmol/l $CaCl_2$ was added to induce MPTP opening (Baines et al., 2003). Absorbance was measured spectrophotometrically at 520 nm for 21 min at 3 min time intervals.

2.10. Materials

All chemicals used were obtained from Sigma Chemical Co.

2.11. Statistical analysis

Statistical analysis of arterial hemodynamic parameters within groups was performed with repeated measures ANOVA followed by Tukey's test and One-way ANOVA followed by Tukey's test was used to determine statistical significance in all other cases. All data were expressed as mean ± S.E.M. Statistical significance was defined as $P<0.05$.

3. Results

3.1. Hemodynamic functions

Tables 1 and 2 demonstrate the time course of heart rate and systolic arterial pressure (SAP) during the experiments. There were no significant differences among groups at baseline before treatment. At the end of 60 min and 120 min reperfusion, heart function was significantly decreased in control group, as indicated by SAP and HR as compared with its baseline. Atractyloside and Cyclosporine A in (AVP+Atr) and (AVP+CsA) groups significantly prevented the decrease in HR at the end of 60 min and 120 min reperfusion compared to their baseline, but had no effect on SAP. SAP not only decreased in CsA group at the end 60 min reperfusion but also significantly increased that compared to control group. As shown in Table 3, Atr and AVP+CsA caused significantly increasing in RPP compared to control. Moreover Atr caused significantly increasing in $-dp/dt$ compared to control. There were no significant differences of intraventricular parameters between any other groups.

3.2. Area at risk and infarct size measurements

There were no significant differences in AAR/LV among groups. Infarct size was 37.6 ± 2.4% in control group, whereas AVP 0.03 μg/rat significantly reduced infarct size to 18.6 ± 1.7 vs. control group (Fig. 2). The reduction in infarct size by AVP 0.03 was abolished by Atractyloside infusion in AVP+Atr group as compared to AVP0.03 (32.5 ± 1.9% vs. 18.6 ± 1.7%) and Cyclosporine A administration in CsA and AVP+CsA groups significantly reduced infarct size to 17.7 ± 4% ($P<0.001$) and 22.7 ± 3% ($P<0.01$) respectively, vs. 37.6 ± 2.4% in control group and returned infarct size as seen as in AVP0.03.

3.3. Biochemical analysis

3.3.1. LDH and CK-MB activity

Compared to control group, administration of AVP 0.03 μg/rat could prevent elevation of LDH activity in plasma after ischemia/reperfusion injury. Also AVP 0.03 significantly reduced CK-MB

Table 3
Intraventricular hemodynamic parameters at 10 min of end reperfusion.

Groups	LVDP	LVEDP	RPP	+dp/dt	−dp/dt
Control	74 ± 5	4.0 ± 0.8	17,539 ± 1784	2029 ± 147	−1638 ± 127
AVP0.03	81 ± 7	1.6 ± 0.4	23,616 ± 2971	2195 ± 254	−1968 ± 236
Atr	90 ± 9	6.2 ± 1.2	33,068 ± 3722[a]	2557 ± 189	−2548 ± 160[b]
AVP+Atr	80 ± 11	3.8 ± 0.3	26,864 ± 4190	2286 ± 325	−2155 ± 317
CsA	96 ± 3	3.8 ± 0.9	28,918 ± 2357	2604 ± 136	−2418 ± 155
AVP+CsA	93 ± 6	4.1 ± 0.8	30,545 ± 2771[b]	2568 ± 159	−2381 ± 165

The values are mean ± S.E.M; AVP, arginine vasopressin; Atr, Atractyloside; CsA, Cyclosporine A; LVDP, left ventricular developed pressure (mmHg); LVEDP, left ventricle end-diastolic pressure (mmHg); RPP, rate pressure product (beats/min mmHg × 10^3); the maximum rise and fall of LV pressures (+dp/dt and −dp/dt, respectively) (mmHg/s).

[a] $P<0.01$ vs. Control.

[b] $P<0.05$ vs. Control.

Table 2
Hemodynamic parameters changes.

Groups	Δ1 Change		Δ2 Change		Δ3 Change	
	HR	SAP	HR	SAP	HR	SAP
Control	−11.04 ± 5.3	−11.74 ± 3.1	−33.64 ± 7.8	−17.1 ± 4	−56.05 ± 10.7	−33.27 ± 5.8
AVP0.03	−7.51 ± 8	−13 ± 3.9	−18.74 ± 11.3	−23.3 ± 7.2	−8.55 ± 17.3	−25.7 ± 4.8
Atr	−22.9 ± 6.2	−8.8 ± 6.7	−25 ± 11.9	−27.1 ± 7.6	−19.8 ± 7.7	−32.4 ± 6.3
AVP+Atr	4.58 ± 8.1	−15.1 ± 8	−16.9 ± 17.7	−27.9 ± 8.9	10.14 ± 11.3	−48.6 ± 12.5
CsA	−0.57 ± 9.3	−22.3 ± 7.2	−12.42 ± 12.4	−21.2 ± 7.9	−29.9 ± 15.7	−37.7 ± 6.3
AVP+CsA	−0.52 ± 13.2	−16.1 ± 2.5	−1.65 ± 7.7	−16.8 ± 4.9	−10.49 ± 12.3	−27.9 ± 3.6

The values are mean ± S.E.M. HR, heart rate (beats/min); SAP, systolic arterial pressure (mmHg); AVP, arginine vasopressin; Atr, Atractyloside; CsA, Cyclosporine A. Δ1 Changes: differences between end of ischemia 30 and end of baseline period, Δ2 changes: differences between end of reperfusion 60 and end of baseline period, Δ3 changes: differences between end of reperfusion 110 and end of baseline period.

level as compared to control group. Atractyloside and Cyclosporine A infusion in AVP+Atr and AVP+CsA groups significantly returned LDH and CK-MB plasma level as seen as in control group (Table 4).

3.3.2. Lipid peroxidation level

MDA plasma level in AVP 0.03 group significantly declined compared to control group. Administration of Atractyloside and Cyclosporine A prior to reperfusion in AVP+Atr and AVP+CsA groups returned MDA plasma level as shown in control (Table 4).

3.4. PAB assay

The PAB value of control group was 46.8 ± 3 (HK unit) (Fig. 3). Compared to control group, administration of AVP 0.03 μg/rat (11.1 ± 0.5 HK, $P<0.001$) could prevent the elevation of PAB value in plasma after ischemia/reperfusion injury and Compared to AVP 0.03 group, Atractyloside and Cyclosporine A in AVP+Atr (45.9 ± 4.6 HK, $P<0.001$) and AVP+CsA (47.4 ± 1.9 HK, $P<0.001$) groups significantly increased PAB value and returned its level as seen as in control group.

3.5. Mitochondrial swelling assays

To determine whether AVP can modulate MPTP opening, we tested the effect of AVP on the decrease of A_{520} in isolated mitochondria. This was used to indicate swelling of the mitochondrion as a result of opening of the pore. At 200 μmol/L, $CaCl_2$, which is known to open the pore, evoked a large decrease in A_{520}. This effect was inhibited by 1 μmol/L CsA, confirming that the decrease in absorbance was due to pore opening. Interestingly, AVP0.03 μg/rat in AVP0.03 group also inhibited the decrease in A_{520}, suggesting that it may protect the heart by inhibiting pore opening (Fig. 4).

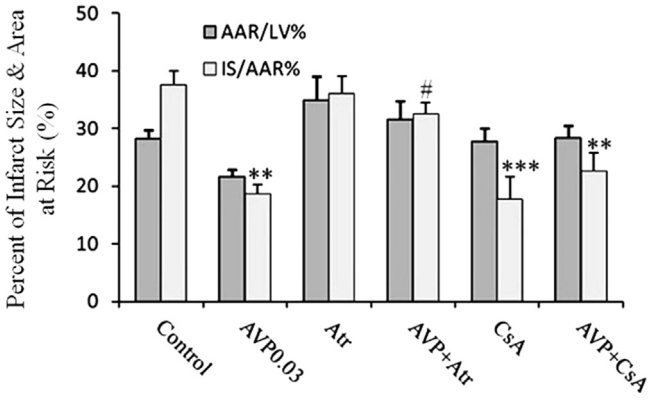

Fig. 2. Myocardial area at risk (AAR/LV %) and infarct size (IS/AAR %) in Control, AVP (0.03 μg/rat), Atr, AVP (0.03 μg/rat)+Atr, CsA and AVP (0.03 μg/rat)+CsA groups. Data are presented as mean ± S.E.M. AVP, arginine vasopressin; Atr, Atractyloside; CsA, Cyclosporine A. $^{**}P<0.01$, $^{***}P<0.001$ vs. control group. $^{\#}P<0.05$ vs. AVP0.03 ($n=13$).

Table 4
The plasma levels of LDH, CK-MB and MDA at the end of reperfusion in Control, AVP (0.03 μg/rat), Atr, AVP (0.03 μg/rat)+Atr, CsA and AVP (0.03 μg/rat)+CsA groups.

Groups	LDH (IU/dl)	CK-MB (IU/dl)	MDA (nmol/ml)
Control	367 ± 23.3	27.9 ± 2.9	4.1 ± 0.2
AVP0.03	182 ± 21.1[a]	9.78 ± 1.2[b]	2.1 ± 0.3[a]
Atr	672 ± 124.8	14.9 ± 1.2[c]	2.7 ± 0.4[a]
AVP+Atr	453 ± 103.5[d]	28.6 ± 7.4[e]	3.0 ± 0.4
CsA	460 ± 66.6	18.8 ± 1.9	3.6 ± 0.6
AVP+CsA	635 ± 106.9[f]	38.7 ± 8.2[f]	3.4 ± 1.4

The values are expressed as mean ± S.E.M; AVP, arginine vasopressin; Atr, Atractyloside; CsA, Cyclosporine A; LDH, lactate dehydrogenase; CK-MB, creatine kinase-MB; MDA, malondialdehyde.

[a] $P<0.01$ vs. Control.
[b] $P<0.001$ vs. Control.
[c] $P<0.05$ vs. Control.
[d] $P<0.05$ vs. AVP0.03.
[e] $P<0.01$ vs. AVP0.03.
[f] $P<0.001$ vs. AVP0.03.

4. Discussion

In the previous work, we showed that AVP can induce preconditioning in a dose-dependent manner via V1 receptor (Nazari et al., 2011).

The present study revealed that exogenous AVP (0.03 μg/rat) can induce preconditioning and significantly decreased infarct size, Biochemical parameters (LDH, CK-MB and MDA plasma levels) and PAB as compared to control group and Administration of Atractyloside (as MPTP opener), decreased the cardioprotective effects of AVP.

It seems that the cardioprotective effect of AVP against myocardial infarction was mediated via inhibiting of MPTP permeability. Many studies have shown a role for mitochondria in reperfusion injury (Rajesh et al., 2003). The mitochondrial permeability transition pore (MPTP) is transmembrane protein that play a major role in both necrotic and apoptotic cell death. The major components of the MPTP are the adenine nucleotide translocase (ANT) in the inner membrane of the mitochondria, cyclophilin D in

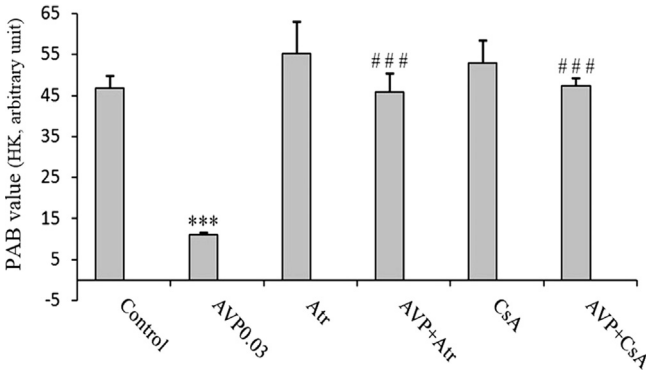

Fig. 3. The PAB value of Control, AVP (0.03 μg/rat), Atr, AVP (0.03 μg/rat)+Atr, CsA and AVP (0.03 μg/rat)+CsA groups. AVP, arginine vasopressin; Atr, Atractyloside; CsA, Cyclosporine A. Data are presented as mean ± S.E.M. $^{***}P<0.001$ vs. control group. $^{\#\#\#}P<0.001$ vs. AVP0.03 group ($n=13$).

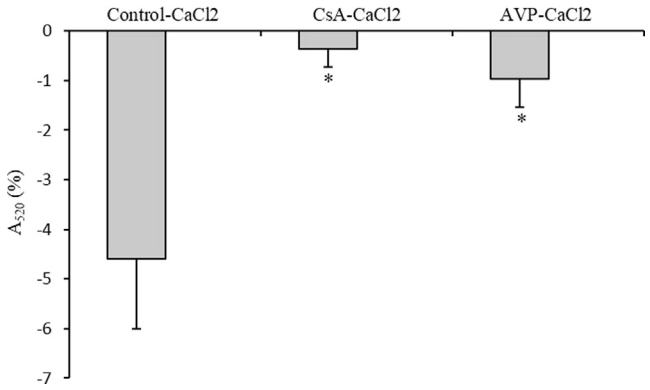

Fig. 4. Percentage reduction in the rate of absorption at 520 nm (A_{520}) in Control–$CaCl_2$, CsA–$CaCl_2$ and AVP–$CaCl_2$ groups in suspensions of rat heart mitochondria exposed to 200 mmol/L $CaCl_2$. Mitochondria from control group that treated with 200 mmol/L $CaCl_2$ served as the Control–$CaCl_2$. Absorbance data 21 min after addition of 200 mmol/L $CaCl_2$. Data are the mean ± S.E.M. $^{*}P<0.05$ compared with Control–$CaCl_2$ ($n=4$).

the matrix and the voltage dependent anion channel (VDAC) in the outer membrane. These proteins are thought to come together at intermembrane junctions to form the MPTP (Halestrap and Brenner, 2003). Under normal physiological conditions, the mitochondrial inner membrane is impermeable. However, under stress, a nonspecific pore known as the MPTP can open in the mitochondrial inner membrane that allows free passage of any molecule of <1.5 kDa (Halestrap et al., 2004). The key factor responsible for MPTP opening is mitochondrial calcium overload, especially when this is accompanied by oxidative stress, adenine nucleotide depletion, elevated phosphate concentrations, and mitochondrial depolarization (Halestrap et al., 2004).

Increased free oxygen radicals cause tissue damage through the peroxidation of the lipids present in the cell membranes and increasing lipid peroxidation might be used as a sign of the tissue damage. MDA is the final product of lipid peroxidation and is used as an indicator of tissue damage caused by oxygen free radicals (Kim et al., 2000; Molina and Garcia, 1997; Sahin et al., 2011). Current data show that plasma level of MDA significantly reduced following exogenous administration of AVP compared to control group moreover caused decrease of the PAB value in favor of the antioxidant status and Atractyloside in AVP+Atr group significantly increased PAB value and returned its level to oxidant status as seen as in control group. A significant amount of evidence is present in the literature to support the role of oxygen free radicals in pathogenesis of I/R injury (Das et al., 1986; Tosaki et al., 1993). This receives further support from the evidence that a variety of free radical scavengers and antioxidants are capable of ameliorating I/R injury (Das and Maulik, 1994). In addition, it has been demonstrated that NO plays a crucial role in cardiac preconditioning. NO production was associated with myocardial preservation during ischemia (Imani et al., 2011; Maulik et al., 1995). Martinez et al. (2003) reported that the modulatory role of NO in the coronary response to AVP may be preserved during partial coronary occlusion in anesthetized goats. Therefore, it seems that AVP via NO production could induce cardioprotection against I/R injury.

Many studies have shown a role for mitochondria in reperfusion injury following ischemia. During reperfusion, cellular calcium increases, resulting in opening of the MPTP and release of cytochrome C. Subsequent activation of caspases results in the failure of ATP generation and mitochondrial membrane potential and leads to cell death (Rajesh et al., 2003). Studies have shown that IPC inhibits a process occurring during ischemia and reperfusion that is responsible for sensitizing the MPTP to calcium and reduced MPTP opening at the onset of reperfusion and exerts its protective effects (Halestrap et al., 2007). So it seems that MPTP is one of the major pathways and main effectors in cardioprotection following precondition period. Atractyloside induces pore formation by ANT, and results in permeabilization of MPTP, causing uncoupling of oxidative phosphorylation (Xu et al., 2001) and abolished the beneficial effect of preconditioning (Hausenloy and Yellon, 2007). The current study has shown that AVP owns a cardioprotective effect via myocyte mitochondria against I/R injury in rat in vivo.

The present study has shown that Cyclosporine A similar to AVP significantly reduced infarct size vs. control group but in other factors included: biochemical parameters (LDH, CK-MB and MDA) and PAB value, we did not see any cardioprotective effects. Moreover CsA caused increase of the PAB value in favor of the oxidant status.

Cyclosporine A (CsA) is a well-known immunosuppressant, but also a potent inhibitor of MPTP opening and is considered to be inherently protective in conditions of ischemia, e.g. in cardiac tissue.

There are several hypotheses to explain the mechanism of CsA-induced adverse effects, including the formation of free oxygen radicals, lipid peroxidation and induction of the cytochrome P450 system (Bianchi et al., 2003; Wang and Salahudeen, 1995; Zachariae, 1999). Several reports have suggested that oxidative stress is the most possible pathway of CsA toxicity (Grieve and Shah, 2003; Rezzani et al., 2003).

CsA due to MPTP inhibition regardless of its oxidant properties has been used in heart ischemia–reperfusion studies and had more beneficial effects (as MPTP inhibitor). In other study CsA (10 mg/kg) was given 10 min prior to ischemia via the femoral vein. CsA significantly reduced infarct size and decreased caspase-3 activity in the myocardium and relieved the injury of mitochondria. CsA reduced the cardiac damage associated with ischemia–reperfusion injury of the heart. The cardioprotective effects of CsA might be associated with the protection of mitochondria and the inhibition of caspase-3 activity (Xie and Yu, 2007).

CsA caused SAP not only decreased at the end 60 min reperfusion but also significantly increased that compared to control group. Rezzani et al. had shown that CsA had adverse effect including increased blood pressure (Rezzani, 2004) that was mediated via increased synthesis of vasoconstrictor eicosanoids (Bianchi et al., 2003). Exogenous AVP in both groups of (AVP+Atr) and (AVP+CsA) prevented the decrease in HR at the end of reperfusion compared to their baseline. It seems that this effect not related to opening and closing of MPTP and probably is done by other pathways that remain to be explored in future studies. 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 modulation of MPTP opening has been observed in cardioprotection by both preconditioning and post-conditioning of the heart (Liu et al., 2008). Mitochondria isolated from AVP–$CaCl_2$ group showed reduced swelling to challenge with 200 μmol/L $CaCl_2$. Furthermore, these mitochondria showed a degree of swelling similar to that induced by CsA. Therefore, it may be suggested that AVP owns a cardioprotective effect against I/R injury by inhibiting MPTP opening. Although the mechanism of the action of AVP in preconditioning responses was not completely explored in my previous (Nazari et al., 2011) and present study, but it had shown that mainly direct activation of V1 receptors on cardiac myocyte can active intracellular signaling specially inhibiting MPTP opening and events previously described (Briley et al., 1994; Dayanithi et al., 2008) similar to preconditioning signaling (Starkopf et al., 1998). Another possibility to consider is that AVP reduced free radical formation in the hearts and finally by inhibiting MPTP opening on cardiac tissue may be the cause of its protection. Thus, AVP may induce preconditioning via several different mechanisms. However, the exact mechanism(s) behind the cardioprotective effects of AVP remain to be explored in future studies.

Further studies are needed to truly disentangle the cardioprotective mechanism of AVP against ischemia–reperfusion injury that included:

a- The cardioprotective effect of endogenous vasopressin (AVP) against ischemia–reperfusion injuries in the anesthetized rat heart.
b- Myocardial expression of V1 receptor in condition of ischemia–reperfusion injuries.
c- Examined the relationship between oxytocin receptors and vasopressin in condition of heart ischemia–reperfusion.
d- The molecular mechanisms of mitochondria responsible for the cardioprotective effect of AVP such as: apoptotic factors expression, myocardial ATP content, the release of cytochrome c, etc.

5. Conclusion

The present study exhibited that exogenous AVP had hormonal preconditioning effect mainly via antioxidant effect and probably by inhibiting MPTP opening on cardiac myocyte against ischemia/reperfusion injury in rat heart in vivo.

Conflict of interest

None declared.

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