The cardioprotective effect of different doses of vasopressin (AVP) against ischemia–reperfusion injuries in the anesthetized rat heart

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A B S T R A C T

The aim of the present study was to investigate the protective effect of various doses of exogenous vasopressin (AVP) against ischemia–reperfusion injury in anesthetized rat heart. Anesthetized rats were randomly divided into seven groups (n=4–13) and all of them subjected to prolonged 30 min regional ischemia and 120 min reperfusion. Group I served as saline control with ischemia, in treatment groups II, III, IV and V, respectively different doses of AVP (0.015, 0.03, 0.06 and 1.2 μg/rat) were infused within 10 min prior to ischemia, in group VI, an AVP-selective V1 receptor antagonist (SR49059, 1 mg/kg, i.v.) was administrated prior to effective dose of AVP injection and in group VII, SR49059 (1 mg/kg, i.v.) was only administrated prior to ischemia. Various doses of AVP significantly prevented the decrease in heart rate (HR) at the end of reperfusion compared to their baseline and decreased infarct size, biochemical parameters [LDH (lactate dehydrogenase), CK-MB (creatinine kinase-MB) and MDA (malondialdehyde) plasma levels], severity and incidence of ventricular arrhythmia, episodes and duration of ventricular tachycardia (VT) as compared to control group. Blockade of V1 receptors by SR49059 attenuated the cardioprotective effect of AVP on ventricular arrhythmias and biochemical parameters, but partially returned infarct size to control. AVP 0.03 μg/rat was known as effective dose. Our results showed that AVP owns a cardioprotective effect probably via V1 receptors on cardiac myocyte against ischemia/reperfusion injury in rat heart in vivo.

1. Introduction

Ischemic myocardial damage is still a serious health problem in the world and today’s, new interventions are designed to decrease cardiomyocyte dysfunction in myocardial ischemia [29]. It has been implied that the valuable effects of reperfusion on the ischemic myocardium might be partially reversed by the occurrence of reperfusion injury and since the reperfusion is initiated by the treatment of myocardial infarction, it is important to limit the extent of this injury [20]. Moreover peroxyl radicals are formed in membranes and lipoproteins as intermediate products of lipid peroxidation, which is related to ischemia reperfusion injury [33]. Preconditioning has been defined as the phenomenon in which usage of pharmacological and physical stimulants prior to ischemia/reperfusion can increase tolerance of ischemic/reperfused heart against myocardial damages. This beneficial effect can be expressed as significant reduction in the width of myocardial cell necrosis, attenuated incidence of reperfusion arrhythmias as well as improved post–ischemic functional recovery of the heart [39]. Several studies have postulated the mechanism underlying the effects of preconditioning on functional and metabolic alterations, including activation of protein kinase C (PKC) and opening K⁺ ATP channels [43]. Vasopressin or arginine vasopressin (AVP), also known as antidiuretic hormone (ADH), is essential for cardiovascular homeostasis [19]. Systemic vasconstriction produced by endogenous AVP appears to be important in blood pressure maintenance [2]. AVP is one of the first described and structurally characterized neuropeptide hormones and, as a result, has been very extensively studied and used clinically over the past 5 decades, mainly to treat hemorrhage and diabetes insipidus [19]. AVP is synthesized as a large prohormone in magnocellular neurons located in the paraventricular and supraoptic nuclei of the hypothalamus [40]. Also AVP is locally produced and released by the heart [21,32]. The release of AVP is stimulated when the plasma osmolality or plasma Na⁺ concentration is increased or the blood pressure is decreased [11]. Vasopressin-receptor subtypes are of the G protein-coupled receptor superfamily and divided to four types including: V1, V2, V3 and OTRs (oxytocin receptor) [19]. V1 receptors (formerly known as V1a receptors) have been found in cardiac myocytes [11] and V1 vascular receptors are located on vascular smooth muscle and mediate vasoconstriction. Hiroyama et al. reported that AVP promotes cardiac hypertrophy in neonatal myocytes. This effect seems to be dependent on the vasopressin V1a receptor (coupled to...
the Gq/G11 signaling pathway) [18]. Several studies have demonstrated a variety of signaling pathways associated with the V1 receptor endogenously expressed in tissues or cell lines. These include activation of phospholipases A2, C, D [3,11] and activation of PKC. The purpose of many investigations has been to find therapeutic approaches to exert preconditioning using pharmacological agents and protect the heart by modulating various signaling pathways with administration of exogenous agent prior to long-lasting ischemia [22]. Regardless of the model used, increasing evidence suggests that stimulation of intracellular signaling events by endogenous agents released during the short, preconditioning ischemia, is important for subsequent cardioprotection [38] and the cardioprotection of ischemic preconditioning is mediated by endogenous active substances, including neurotransmitters and autacoids [1]. Some studies have considered the possible relation between hormones and preconditioning [16,31,37]. Since, AVP may play an important physiological role in regulation of vascular tone and cardiac function and V1 receptors stimulation on cardiac myocyte can active intracellular signaling events similar to preconditioning signaling, therefore the present study was designed to analyze the possible protective effect of AVP against regional ischemic reperfusion (1R) injury in heart, to determine the dose–response relationship of AVP on its cardioprotective effect and to investigate the role of AVP specific receptors (V1) in cardioprotection in anesthetized rats.

2. Materials and methods

Male rats (weighing 280–310 g) housed in the animal quarters of the Tehran University of medical sciences 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 ad libitum. 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 body weight, i.p.) and maintained with supplemental doses (~30 mg/kg) every 60–70 min, as needed. Body temperature was measured by rectal thermometer and maintained at 37 ± 1°C. After a tracheotomy in the middle of the neck and tracheal intubation, 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 dye and other drugs. A standard limb 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, ADInstruments). 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 [36].

Rats were given heparin (200 IU/kg, i.v.), and then the chest was opened by a left thoracotomy in the fourth rib approximately 3 mm from the sternum 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 immediately the left atrial appendage to the right part of the LV. Both ends of the silk thread were passed through coronary ligation. 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 following coronary artery occlusion, and reperfusion was achieved by releasing the tension on the ligature. Ischemia was confirmed by ST elevation and increase in R-wave amplitude in ECG, or cardiac cyanosis subsequent decrease in blood pressure, and reperfusion was confirmed by epicardial hyperemia. At the end of the surgical procedure, any rat with a constant fall in blood pressure to less than 70 mmHg was discarded from the study.

2.2. Experimental protocol

This study included of three protocols. Rats were randomly divided into seven groups (n = 4–13) and all of them subjected to 30 min ischemia and 120 min reperfusion (Fig. 1). After a stabilization period following the surgical preparation, basal hemodynamic parameters were measured for 15 min before drug administration. In protocol I (control group), saline was administered intravenously before ischemia. In protocol II, different doses of AVP (0.015, 0.03, 0.06 and 1.2 µg/rat) were given as an i.v. infusion 10 min before ischemia. In protocol III SR49059 (1 mg/kg, i.v.), as an AVP-selective V1 receptor antagonist, was injected 20 min prior to ischemia with and without the effective dose of AVP (0.03 µg/rat) into two different groups.

2.3. Hemodynamic functions

Arterial blood pressure and HR were continuously monitored and recorded throughout the experimental protocols. Left ventricular hemodynamic parameters such as: left ventricular end-diastolic pressure (LVEDP), left ventricular developed pressure (LVPD = LVSVP (left ventricular systolic pressure) – LVEDP), maximum rise and fall of LV pressures (+dp/dt and –dp/dt, respectively) and RPP (rate pressure product = LVPD × 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 for area at risk (AAR) determination. The Evans blue solution stains the perfused myocardium, while the occluded vascular bed remains unstained. Then, the heart was excised, and both atria and the roots of the great vessels were removed. The heart was frozen overnight and then cut into slices of 2-mm-thick. All slices were incubated with a 1% solution of 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. Determination of arrhythmia scores

During the 30-min ischemia, ventricular arrhythmias were evaluated according to Lambeth convention [42]. Ventricular ectopic beats (VEBs) were defined as identifiable premature QRS complexes. Ventricular tachycardia (VT) was defined as a run of four or more ventricular premature beats and ventricular fibrillation (VF) was defined as a signal for which individual QRS deflections can no longer be distinguished from one other and for which a rate can no longer be measured. Original ECG recordings are illustrated in Fig. 2. The incidence, onset time and duration of arrhythmias were assigned to identify arrhythmias severity according to the following scoring system [8].
Fig. 1. Illustration of the experimental protocols. Animals in control group (protocol I) were subjected to 30 min ischemia followed by 120 min reperfusion. In protocol II, AVP (arginine vasopressin) groups were given i.v. infusions of different doses of AVP (0.015, 0.03, 0.06 and 1.2 µg/rat) 10 min before ischemia. In protocol III, SR49059 (1 mg/kg, i.v.), as an AVP-selective V1 receptor antagonist, was injected 20 min prior to ischemia with and without AVP (0.03 µg/rat) as the effective dose in two different groups. NS = normal saline, TTC = triphenyltetrazolium chloride.

Fig. 2. Electrocardiogram recording. (A) During baseline; (B) during coronary artery occlusion; (C) ventricular ectopic beat (VEB); (D) couplet; (E) bigeminy; (F) ventricular tachycardia (VT); (G) ventricular fibrillation (VF).

0: <10 ventricular premature beats, 1: ≥10 ventricular premature beats, 2: VT (duration <30 s), 3: VT (duration ≥30 s), 4: VF starting 15 min after the onset of ischemia, 5: VF starting 5–15 min after the onset of ischemia, 6: VF starting within 5 min after the onset of ischemia.

2.6. Biochemical analysis

Blood samples were collected at the end of reperfusion for measurement of the cardiac enzymes, including the activity of creatine kinase-MB isoenzyme (CK-MB), lactate dehydrogenase (LDH) and
malondialdehyde (MDA) content as a redox estimation. The heparinized samples were centrifuged at 5000 rpm, 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. [35].

2.7. Materials

AVP, 2,3,5-triphenyltetrazolium chloride and Evans blue were obtained from Sigma Chemical Co. and SR49059 from Tocris Bioscience Co.

2.8. Statistical analysis

Statistical analysis of arterial hemodynamic parameters within groups was performed with repeated measures ANOVA followed by the Tukey’s test. Differences in intraventricular hemodynamic parameters, infarct size, CK-MB, LDH and MDA plasma content were determined by one-way ANOVA followed by the Tukey’s test. Arrhythmia scores were analyzed with Kruskal–Wallis test, and the incidences of VT or VF were compared by Fisher exact test. All data were expressed as mean ± SEM. Statistical significance was defined as P<0.05.

3. Results

3.1. Hemodynamic functions

Table 1 demonstrates 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. AVP 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. As shown in Table 2, AVP 0.015 caused significantly increasing in RPP and −dp/dt and also AVP 0.03 significantly decreased LVEDP compared to control. There were no significant differences of intraventricular parameters between any other groups.

3.2. Area at risk and infarct size measurements

Fig. 3A shows the original pictures of staining heart. The area at risk (AAR/LV) and the infarct size (IS/AAR) are shown in Fig. 3B. There were no significant differences in AAR/LV among groups. Infarct size was 37.6 ± 2.4% in Control group, whereas administration of different doses of vasopressin (AVP 0.015, 0.03, 0.06 and 0.12 µg/rat) significantly reduced infarct size to 25.9 ± 1.4%, 18.6 ± 1.7%, 18 ± 1.8% and 21.7 ± 1.8% respectively vs. control group. The reduction in infarct size by AVP 0.03 was partially abolished by pretreatment with SR49059 in SR+AVP group as compared to AVP0.03 (28.8 ± 1.4% vs. 18.6 ± 1.7%).

3.3. Ventricular arrhythmias during ischemia

3.3.1. Severity of arrhythmias

Administration of AVP (0.015 and 0.03) prior to ischemia significantly diminished ventricular arrhythmias severity compared to control group (2.2 ± 0.4 and 2.3 ± 0.2, respectively, vs. 3.6 ± 0.3 in control group). Administration of SR49059 in SR+AVP group intensified severity of arrhythmias compared to AVP0.03 group (4.3 ± 0.5 vs. 2.3 ± 0.2 in AVP0.03) and abolished the cardioprotective effect of vasopressin on arrhythmias (Fig. 4).

3.3.2. Incidence of VT and VF

In all groups, rats were experienced VT, while VF occurred in some hearts in each groups. In control group, VF occurred in 42% of hearts. Only, VF incidence was attenuated in AVP0.015 (17%) compared to control. Administration of SR49059 in SR+AVP and SR groups increased the incidence of VF respectively to 71% and
Table 1
Hemodynamic parameters. The values are mean ± SEM; HR = heart rate (beats/min); SAP = systolic arterial pressure (mmHg); AVP = arginine vasopressin; SR = V1 selective antagonist SR49059.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Baseline</th>
<th>End of ischemia 30'</th>
<th>End of reperfusion 60'</th>
<th>End of reperfusion 120'</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR</td>
<td>SAP</td>
<td>HR</td>
<td>SAP</td>
</tr>
<tr>
<td>Control</td>
<td>308 ± 12</td>
<td>109 ± 5</td>
<td>290 ± 15</td>
<td>97 ± 5</td>
</tr>
<tr>
<td>AVP0.015</td>
<td>362 ± 24</td>
<td>130 ± 7</td>
<td>334 ± 30</td>
<td>111 ± 10</td>
</tr>
<tr>
<td>AVP0.03</td>
<td>312 ± 18</td>
<td>110 ± 7</td>
<td>304 ± 14</td>
<td>97 ± 5</td>
</tr>
<tr>
<td>AVP0.06</td>
<td>333 ± 10</td>
<td>112 ± 8</td>
<td>324 ± 15</td>
<td>112 ± 6</td>
</tr>
<tr>
<td>AVP0.12</td>
<td>348 ± 11</td>
<td>119 ± 8</td>
<td>348 ± 11</td>
<td>109 ± 6</td>
</tr>
<tr>
<td>SR + AVP</td>
<td>337 ± 9</td>
<td>131 ± 7</td>
<td>335 ± 11</td>
<td>103 ± 9**</td>
</tr>
<tr>
<td>SR</td>
<td>338 ± 17</td>
<td>128 ± 2</td>
<td>352 ± 28</td>
<td>116 ± 6</td>
</tr>
</tbody>
</table>

* P < 0.05 vs. its baseline within group.
** P < 0.01 vs. its baseline within group.
*** P < 0.001 vs. its baseline within group.

Table 2
Intraventricular hemodynamic parameters at 10 min of end reperfusion. The values are mean ± SEM; AVP = arginine vasopressin; SR = V1 selective antagonist SR49059; LVDP = left ventricular developed pressure (mmHg); LVEDP = left ventricle end-diastolic pressure (mmHg); RPP = rate pressure product (beats/min mmHg x 10^3); the maximum rise and fall of LV pressures (+dp/dt and –dp/dt, respectively) (mmHg/s).

<table>
<thead>
<tr>
<th>Groups</th>
<th>LVDP</th>
<th>LVEDP</th>
<th>RPP</th>
<th>+dp/dt</th>
<th>–dp/dt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>74 ± 5</td>
<td>4.0 ± 0.8</td>
<td>17539 ± 1784</td>
<td>2029 ± 147</td>
<td>–1638 ± 127</td>
</tr>
<tr>
<td>AVP0.015</td>
<td>98 ± 6</td>
<td>2.9 ± 0.4</td>
<td>33790 ± 5369*</td>
<td>2855 ± 203</td>
<td>–2874 ± 276***</td>
</tr>
<tr>
<td>AVP0.03</td>
<td>81 ± 7</td>
<td>1.6 ± 0.4</td>
<td>23616 ± 2971</td>
<td>2195 ± 254</td>
<td>–1968 ± 236</td>
</tr>
<tr>
<td>AVP0.06</td>
<td>80 ± 7</td>
<td>2.5 ± 0.3</td>
<td>23420 ± 4287</td>
<td>2228 ± 172</td>
<td>–2056 ± 172</td>
</tr>
<tr>
<td>AVP0.12</td>
<td>79 ± 3</td>
<td>2.3 ± 0.2</td>
<td>23292 ± 1563</td>
<td>2270 ± 96</td>
<td>–2150 ± 75</td>
</tr>
<tr>
<td>SR + AVP</td>
<td>83 ± 9</td>
<td>2.6 ± 0.4</td>
<td>26880 ± 4090</td>
<td>2349 ± 234</td>
<td>–2117 ± 260</td>
</tr>
<tr>
<td>SR</td>
<td>65 ± 6</td>
<td>2.5 ± 0.3</td>
<td>19616 ± 1487</td>
<td>1948 ± 231</td>
<td>–2150 ± 75</td>
</tr>
</tbody>
</table>

* P < 0.05 vs. control.
** P < 0.01 vs. control.

75% (vs. 33% in AVP0.03). Moreover these values are significantly higher than control group (Fig. 5).

3.3. Number of episodes and duration of VT
The mean number of VT episodes during 30 min of ischemia in AVP0.03 group was markedly reduced, compared with control group (7 ± 1 vs. 24 ± 3 in control rats). Treatment with SR49059 in SR + AVP group, enhanced the number of VT episodes, compared to AVP0.03 and alone in SR group enhanced the number of VT episodes, compared to control (Fig. 6). Compared with control rats, duration of VT throughout 30 min of ischemia was significantly reduced by administration of vasopressin in all of AVP (0.015, 0.03, 0.06 and 0.12) groups (respectively, 10 ± 5, 27 ± 8, 27 ± 9, 21 ± 7 s vs. 85 ± 10 s in control rats) (Fig. 6).
Table 3

<table>
<thead>
<tr>
<th>Groups</th>
<th>LDH (U/dl)</th>
<th>CK-MB (U/dl)</th>
<th>MDA (nmol/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>367 ± 23.3</td>
<td>29.1 ± 3.4</td>
<td>4.1 ± 0.2</td>
</tr>
<tr>
<td>AVP0.015</td>
<td>257 ± 29.0</td>
<td>28.5 ± 2.5</td>
<td>3.8 ± 0.4</td>
</tr>
<tr>
<td>AVP0.03</td>
<td>182 ± 21.1</td>
<td>7.98 ± 1.2</td>
<td>2.1 ± 0.3</td>
</tr>
<tr>
<td>AVP0.06</td>
<td>192 ± 45.5</td>
<td>17.3 ± 2.2</td>
<td>2.0 ± 0.3</td>
</tr>
<tr>
<td>AVP0.12</td>
<td>529 ± 31.7</td>
<td>42.4 ± 3.8</td>
<td>3.4 ± 0.4</td>
</tr>
<tr>
<td>SR + AVP</td>
<td>315 ± 21.0</td>
<td>31.9 ± 5.9</td>
<td>3.7 ± 0.3**</td>
</tr>
<tr>
<td>SR</td>
<td>388 ± 85.5</td>
<td>30.5 ± 3.9</td>
<td>3.3 ± 0.2**</td>
</tr>
</tbody>
</table>

*P<0.01 vs. control.
**P<0.001 vs. control.
***P<0.05 vs. AVP0.03.

3.4. Biochemical analysis

3.4.1. LDH and CK-MB activity

In protocol II, compared to control group, administration of AVP0.03, 0.06 and 0.12 μg/rat could prevent elevation of LDH activity in plasma after ischemia/reperfusion injury. Also AVP0.03 significantly reduced CK-MB level as compared to control group. In protocol III, SR49059 injection prior to AVP in SR + AVP group significantly returned LDH and CK-MB plasma level as seen as in control group (Table 3).

3.4.2. Lipid peroxidation level

MDA plasma level in AVP0.03 and 0.06 treatment groups significantly declined compared to control group. Administration of SR49059 prior to AVP0.03 returned MDA plasma level as shown in control (Table 3).

3.5. Dose–response studies

Vasopressin decreased post-ischemic infarct size, LDH, CK-MB and MDA in a U-shaped manner. The optimum dose of AVP which had these effects was 0.03 μg/rat.

4. Discussion

The present study revealed that exogenous AVP had hormonal preconditioning effect probably via V1 vasopressin receptor on cardiomyocyte against IR injury. To our knowledge, this is the first experimental evidence showing that AVP can induce preconditioning, AVP significantly decreased infarct size. Biochemical parameters (LDH, CK-MB and MDA plasma levels), severity and incidence of ventricular arrhythmia and episodes and duration of VT as compared to control group. Administration of V1 receptor antagonist decreased the cardioprotective effects of AVP.

Our data reveals that the treatment with exogenous AVP before regional myocardial ischemia reperfusion can provide myocardial protection against reperfusion injury by decreasing infarct size. The present in vivo observations are inconsistent with previous study, which indicated that AVP concentrations were significantly positively correlated with infarct size [12,14]. It is generally accepted that AVP activates two types of receptors, V1 vasopressin and OT (oxytocin) receptors on the heart [7]. Blockage of V1 receptor by SR49059, partially returned infarct size to control. It seems that the cardioprotective effect of AVP against myocardial infarction was mediated via activation two types of AVP receptors on the heart that needed more investigation. In addition in our study, administration of V1 receptor antagonist alone did not affect infarct size compared to control group, indicating that endogenous AVP had no effect against myocardial infarction.

Occlusion of LAD led to a fall in blood pressure, therefore it seems that coronary flow can be affected by reduced blood pressure in the unoccluded myocardial area, becoming a potential trigger for additional arrhythmia. Reduction in infarct size correlate well with the antiarrhythmic effects of AVP. Therefore, it appears that the extent of myocardial infarction may affect directly arrhythmia. In our study, various doses of exogenous AVP had antiarrhythmic effects and injection of V1 selective antagonist before AVP markedly intensified the ventricular arrhythmias indicated that antiarrhythmic effects of AVP are mediated via V1 receptor. It has been suggested that β-adrenergic responsibility significantly contributes to the arrhythmogenesis and has a significant role in the pathogenesis in ischemic myocardium [23,24]. Guideri et al. had shown that endogenous AVP may also suppress arrhythmogenic activity elicited by beta-adrenergic receptor stimulation [17]. Thus, it is possible that β-adrenergic suppression may be mediated by V1 activation since, injection of V1 selective antagonist alone markedly increased the ventricular arrhythmias compared to control, it seems that exogenous and endogenous AVP exerts cardioprotective effect against ventricular arrhythmias.

In addition, AVP reduced biochemical parameters in our experiment. CK-MB and LDH being the myocardial enzymes, leak out from the tissue to plasma on development of degenerative changes in myocardial cell membranes [30]. Elevated levels of CK-MB isoenzyme have been considered as important markers of myocyte injury [44] and we observed that when AVP administrated before ischemia could induce a significant reduction in the level of this enzyme compared to control group. Increase in plasma LDH level, which plays an important role in systemic tissue damage [13], was also attenuated by vasopressin treatment. In support of the current data, other study confirmed that each cardioprotective agent could reverse elevated plasma of CK-MB and LDH in ischemic/reperfused heart [20]. Thus reduction in biochemical parameters due to AVP injection confirms the cardioprotective effect of it against ischemia/reperfusion-induced injuries.

Free oxygen radicals are produced in all body cells in a limited number under normal conditions and are neutralized by endogenous antioxidants agent. 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. Measurement of the MDA level in serum is used as an indicator of tissue damage caused by in vivo free oxygen radicals [25,28,34]. Current data show that plasma level of MDA significantly reduced following exogenous administration of some doses of AVP compared to control group. A significant amount of evidence is present in the literature to support the role of oxygen free radicals in pathogenesis of myocardial ischemia reperfusion injury [3,41]. This receives further support from the evidence that a variety of free radical scavengers and antioxidants are capable of ameliorating ischemia reperfusion injury [10]. In addition, it has been demonstrated that nitric oxide plays a crucial role in cardiac preconditioning. NO production was associated with myocardial preservation during ischemia while inhibition of NO synthesis enhanced myocardial ischemia reperfusion injury [27]. Martinez et al. reported that the modulatory role of nitric oxide in the coronary response to AVP may be preserved during partial coronary occlusion in anesthetized goats [26]. Therefore, it seems that AVP via NO production could induce cardioprotection against I/R injury. In present study, a marked fall in SAP and HR was observed in control group at the end of reperfusion as compared with its baseline. Exogenous AVP and SR49059 prevented the decrease in HR at the end of reperfusion compared to their baseline, but had no effect on SAP. Vasopressin is a weak vasopressor in animals with an intact autonomic nervous system because it causes leftward shift of the heart rate-arterial pressure baroreflex curve by acting on...
V1 receptors in the brain as explained previously. As a result, the hypertensive effects of vasopressin are diminished because vasopressin causes a reduction in heart rate greater than that observed with other vasoconstrictors [19].

The decreased HR in present study at the end of reperfusion in control group was in line with previous study reporting that endogenous arginine vasopressin (AVP) during hypoxia, ischemia, and severe hemorrhage, acting within the central nervous system primarily on V1 receptors at the area postrema, enhances both cardiopulmonary and arterial baroreflex function [6] and resembles the properties of β-blockers [30]. Thus, endogenous AVP may be induced bradycardia. And in our study, blockade of V1 receptors abolished this bradycardia effect [4].

In contrary to endogenous AVP, injection of exogenous AVP (0.03 and 0.06), maintained the heart rate at reperfusion period to baseline levels. In support of the current data, Chiba [5] reported that vasopressin had a direct stimulating effect on sinoatrial nodal pacemaker activity and a direct suppressive effect on atrial contractility. Furukawa et al. [15] also reported that vasopressin induced a small increase in the sinus rate in isolated perfused dog heart preparations. Therefore, it may be supposed that exogenous vasopressin may exert different cardiovascular effects than those mediated via naturally central released endogenous vasopressin. Since, SR49059 alone could not decrease infarct size, LDH, CK-MB and MDA levels to normal, thus it seems that restoration of HR effect could not induced cardioprotection against I/R injury. This observation suggests that the effects of AVP preconditioning on myocardial infarction are not related to alterations in hemodynamic parameters. Since, AVP in some doses significantly improved RPP, LVEDP and −dp/dt, it seems that AVP had a beneficial effect on left ventricular function.

A reasonable rationale for clinical using would have been to use vasopressin in a low dose, as an additional therapy. Use of low-dose vasopressin in patients with severe septic shock potentially avoided renal, mesenteric, pulmonary, and coronary ischemia was shown in previous study [19]. In this study vasopressin treatment reveals a biphasic pattern, i.e., increasing doses correlate with the increasing efficacy of injury inhibition until an optimal dose is attained beyond which higher doses show less activity. The lowest AVP dose with optimal effect in the reuction of the infarct size, biochemical changes and hemodynamic improvement was induced by i.v. infusion of 0.03 μg/rat vasopressin. For all indices of protection, higher and lower doses of AVP than 0.03 μg/rat revealed a less protective effect. This form of the effect of AVP has been shown by other agent such as oxytocin [20].

Although the mechanism of the action of AVP in preconditioning responses was not explored in this study, it had shown that probably direct activation of V1 receptors on cardiac myocyte can active intracellular signaling events previously described [3] similar to preconditioning signaling [38]. Another possibility to consider is that AVP reduced free radical formation in the hearts by NO production via heart V1 receptor activation. And antioxidiant effects of AVP 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.

5. Conclusion

The administration of AVP before regional myocardial ischemia and reperfusion reduced the extent of irreversible myocardial injury by a mechanism probably involving activation of the heart V1 receptors.