Journal of Cardiology 61 (2013) 79–86

Contents lists available at SciVerse ScienceDirect

Journal of Cardiology

journal homepage: www.elsevier.com/locate/jjcc

Original article

The role of central oxytocin in stress-induced cardioprotection in ischemic-reperfused heart model

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ARTICLE INFO

Article history:
Received 13 February 2012
Received in revised form 23 June 2012
Accepted 6 August 2012
Available online 16 November 2012

Keywords:
Stress
Oxytocin
Ischemia/reperfusion
Heart
Receptor

ABSTRACT

Background and purpose: There is growing evidence that stress contributes to cardiovascular disease and triggers the release of oxytocin. Moreover previous studies confirmed oxytocin mimics the protection associated with ischemic preconditioning. The present study was aimed to assess the possible cardioprotective effects of the centrally released oxytocin in response to stress and intracerebroventricular (i.c.v.) administration of exogenous oxytocin in ischemic-reperfused isolated rat heart.
Methods and subjects: Rats were divided in two main groups and all of them were subjected to i.c.v. infusion of vehicle or drugs: unstressed rats [control: vehicle, oxytocin (OT; 100 ng/5 μl), atosiban (ATO; 4.3 μg/5 μl) as oxytocin antagonist, ATO + OT] and stressed rats [St: stress, OT + St, ATO + St]. After anesthesia, hearts were isolated and subjected to 30 min regional ischemia and 60 min reperfusion (IR). Acute stress protocol included swimming for 10 min before anesthesia. Myocardial function, infarct size, coronary flow, ventricular arrhythmia, and biochemical parameters such as creatine kinase and lactate dehydrogenase were measured. Ischemia-induced ventricular arrhythmias were counted during the occlusion period.
Results: The plasma levels of oxytocin and corticosterone were significantly elevated by stress. Unexpectedly hearts of stressed rats showed a marked depression of IR injury compared to control group. I.c.v. infusion of oxytocin mimicked the cardioprotective effects of stress, yet did not elevate plasma oxytocin level. The protective effects of both stress and i.c.v. oxytocin were blocked by i.c.v. oxytocin antagonist.
Conclusions: These findings suggest that i.c.v. infusion of exogenous oxytocin and centrally released endogenous oxytocin in response to stress could play a role in induction of a preconditioning effect in ischemic-reperfused rat heart via brain receptors.

Introduction

Intensive cardiovascular research is set to identify a reliable cardioprotective intervention that can salvage ischemic myocardium.

Cardiac preconditioning represents the most potent and consistently reproducible method of rescuing heart tissue from undergoing irreversible ischemic damage. Several recent clinical studies documented the significant role of stress in evoking the severity of cardiovascular disease [1]. In this regard, heart failure has been recognized as an autonomic nervous system dysfunction [2] and increasing parasympathetic and decreasing sympathetic nervous activity in patients with chronic heart failure improved cardiac function [3]. Moreover, as a neurohormone and as a neurotransmitter, oxytocin has been involved in the stress response [4] and is well known to exert potent physiological anti-stress effects [5]. Oxytocin has also been implicated in the cardiovascular response to physical exercise and stress adjustments [1,6,7]. However, physiological functions of oxytocin released during stress are not well understood. Regarding other stress hormones, oxytocin was found to influence catecholamine release [8].

More recently, oxytocin has been considered to be a cardiovascular hormone [9]. Anatomical studies of oxytocin pathways in the brain have revealed extensive innervation of the brain stem structures regulating the cardiovascular, behavioral, and neuroendocrine responses to stress by oxytocin fibers projecting from the paraventricular nucleus (PVN) [10]. Expression of oxytocin receptors in the same regions of the brain stem has been also well documented [11].

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0914-5087/$ – see front matter
http://dx.doi.org/10.1016/j.jjcc.2012.08.021

Some investigators have provided evidence that oxytocin may be involved in regulation of the cardiovascular system by means of direct peripheral and indirect central actions [12]. Our previous studies confirmed the peripherally protective effects of oxytocin on myocardial injury of the ischemic reperfused heart in the anesthetized rat [13,14]. Moreover exposure to various stressors such as swim stress (10 min, 19–21 °C) a combined emotional and physical stressor triggered the release of oxytocin within both supra optic nucleus (SON) and PVN, as studied in male and female rats [15,16], which paralleled oxytocin secretion into blood [15,17].

The engagement of central oxytocin in the controlling neuroendocrine responses to stress, its putative contribution to the regulation of cardiovascular parameters, and its protective effect on ischemia/reperfusion-induced myocardial injury, raises the question whether central release of oxytocin in response to stress and central administration of exogenous oxytocin may also be involved in regulation of the cardiovascular system in ischemic-reperfused heart.

Therefore the present study was designed to evaluate the possible cardioprotective effects of oxytocin released into brain in response to stress and central administration of exogenous oxytocin on ischemic-reperfused isolated rat heart.

Materials and methods

Animals

Male Wistar rats (200–250 g) were obtained from Tehran University of Medical Sciences and were housed in an air-conditioned colony room on a light/dark (12/12 h) cycle (light on at 7 am) at 21–23 °C with free access to food and water. The rats were housed individually in stainless steel cages and anesthetized with sodium pentobarbital (60 mg/kg, 15 mg/0.5 ml, i.p.) and given heparin sodium (500 IU/0.5 ml, i.p.). All experiments were conducted in accordance with the institutional guidelines of Tehran University of Medical Sciences (Tehran, Iran) and the National Institutes of Health guidelines for the care and use of laboratory animals.

Implantation of the intracerebroventricular guide tube

Rats were deeply anesthetized with i.p. injection of a mixture of ketamine (100 mg/kg) and xylazine (5 mg/kg) and then placed on a stereotaxic device. A 23-gauge stainless steel thin wall cannulae (9 mm long), was implanted into the lateral ventricle (intracerebroventricular; i.c.v.) using the following stereotaxic coordinates: 0.8 mm posterior to the bregma and 1.5 mm lateral from the midline, 3.2 mm below the surface of the skull. The guide tube was fixed to the skull using two stainless screws and dental acrylic cement and closed with a stainless steel stylet (30G). After surgery, the rats received antibiotic (penicillin 6.3.3., 30,000 U in 1 ml/rat i.m.) and were placed in their home cages. The animals were allowed 1 week of recovery after surgery.

At the beginning of each experimental session, the stylet was removed from the guide tube and the stainless steel tube (30G) that was 0.1 mm longer than the stylet was inserted into the guide tube and connected via the polyvinyl tubing to a micro syringe placed in an infusion pump (NE-1000, New Era Pump Systems Inc., Farmingdale, NY, USA). Each i.c.v. infusion was delivered at the rate of 5 μl/h. Rats received i.c.v. infusion of vehicle or drugs before anesthesia.

Forced swimming

For stress induction, the rats were forced to swim for 10 min in deep water at 19–20 °C at 09:00 am. The forced swimming apparatus consisted of a Plexiglas cylinder that was 50 cm high and 30 cm in diameter. The cylinder was filled with tap water to a height of 35 cm. Rats were transferred to the cylinder from their home cages, forced to swim in the apparatus for 10 min and returned to their home cage [18,19].

Preparation of isolated hearts

After anesthesia hearts were rapidly excised and placed in an ice-cold buffer, and mounted on a constant pressure (80 mmHg) Langendorff-perfusion apparatus.

Hearts were perfused retrogradely with modified Krebs–Henseleit bicarbonate buffer containing (in mmol/l): $NaHCO_3$ 25; KCl 4.7; NaCl 118.5; $MgSO_4$ 1.2; KH_2PO_4 1.2; glucose 11; $CaCl_2$ 2.5 gassed with 95% O_2/5% CO_2 (pH 7.35–7.45 at 37 °C). A latex, fluid-filled, isovolumic balloon was inserted into the left ventricle through the left atrial appendage and inflated to give a diastolic pressure of 5–7 mmHg and connected to a pressure transducer (Harvard Apparatus, Holliston, MA, USA). To assess ventricular arrhythmia and heart rate monitoring, electrocardiographic recording was performed by fixation of thin stainless steel electrodes on ventricular apex and right atrium. A surgical needle (6-0 silk suture) was passed under the origin of the left anterior descending coronary artery, and the ends of the suture were passed through two plastic pipette tips to form a snare. Regional ischemia was induced by tightening the snare and reperfusion was performed by releasing the ends of the suture. The perfusion apparatus was water-jacketed to maintain constant perfusion temperatures of 37 °C. Hearts were allowed to beat spontaneously throughout the experiments. Hemodynamic parameters [left ventricular developed pressure (LVDP, the difference between left ventricular systolic and diastolic pressure) and heart rate (HR)] were monitored with a homemade program (Ossilo Graph Monitor, Biomed, Tehran, Iran). Left ventricular function was assessed by the rate pressure product (HR × LVDP). Ischemia-induced ventricular arrhythmias were counted during the occlusion period. Coronary effluent was collected at the end of reperfusion to measure enzymes including creatine kinase-MB (CK-MB) and lactate dehydrogenase (LDH) as biochemical markers of myocyte necrosis.

Experimental protocol

Rats were randomly divided in two main stressed and non-stressed groups, and received i.c.v. infusion of vehicle or drugs before anesthesia and isolated hearts were subjected to 30 min ischemia and 60 min reperfusion.

Unstressed groups included: (1) control ($n = 7$), rats received vehicle; (2) OT ($n = 7$), oxytocin (OT) (100 ng/5 μl/h) was used; (3) ATO + OT ($n = 5$), atosiban (ATO) was administered (4.3 μg/5 μl/h) prior to infusion of oxytocin; (4) ATO ($n = 6$); atosiban was infused. Stressed groups exposed to swim stress for 10 min before anesthesia include: (1) St (stress) ($n = 5$), rats received vehicle 10 min prior to stress; (2) ATO + St ($n = 5$), atosiban was infused 10 min prior to stress; (3) OT + St ($n = 6$), oxytocin was infused 10 min prior to stress (Fig. 1).

Infarct size measurement

After completion of the reperfusion period, the left coronary artery was reoccluded, and Evans blue dye was infused via the aorta to differentiate the ischemic zone (area at risk; AAR) from the non-ischemic zone. Hearts were frozen overnight and then sliced into 2.0 mm (using stainless steel rat heart slicer matrix with 2.0 mm coronal section slice intervals) transverse sections from apex to base. Slices were then incubated with 1% triphenyl tetrazolium chloride (TTC in 0.1 M phosphate buffer, pH 7.4) for a period of 20 min at 37 °C. TTC reacts with viable tissue, producing a red

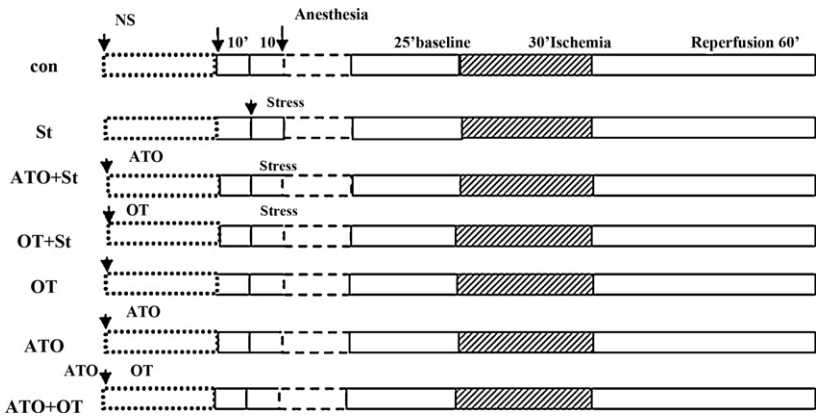

Fig. 1. Illustration of the experimental protocols. Hearts in all groups were subjected to 30 min of ischemia followed by 60 min reperfusion. con, control; St, stress; OT, oxytocin; ATO, atosiban; NS, normal saline.

formazan derivative, which is distinct from the white necrotic tissue once fixed in 10% formalin for 24 h. The areas of the left ventricle, AAR, and infarcted tissues were measured by method of planimetry from the scanned hearts by using Photoshop program. AAR was expressed as a percentage of left ventricular size for each heart and the infarct size was expressed as a percentage of AAR [20–22].

Biochemical analysis

The coronary effluent was collected for cumulative CK and LDH release at 60 min of reperfusion. LDH and CK concentration in perfusate were determined by an autoanalyzer (Roche Hitachi Modular DP Systems, Mannheim, Germany) using specific kit (Sigma Chemical Co., St. Louis, MO, USA).

Assessment of ventricular arrhythmias

Ischemia-induced ventricular arrhythmias were determined in accordance with the Lambeth Conventions [23]. In this regard, three forms of ventricular arrhythmias were analyzed as below: ventricular ectopic beat (VEB), was identified as premature QRS complex; ventricular tachycardia (VT) was defined as four or more serial VEBs; and ventricular fibrillation (VF), was characterized as undetectable QRS complex. Multipart forms of VEBs such as bigeminy and salvos (couplet and triplet) were counted as separate episodes. The incidence, time of occurrence, and duration of arrhythmias were used to identify arrhythmia severity according to the following scoring system [24]: 0: 0–49 VEBs; 1: 50–499 VEBs; 2: >499 VEBs and/or 1 episode of spontaneously reverting VT or VF; 3: >1 episode of VT or VF or both with a total duration <60 s; 4: VT or VF or both 60–120 s total duration; 5: VT or VF or both >120 s duration; 6: fatal VF starting at >15 min after occlusion; 7: fatal VF starting between 4 and 14 min 59 s; 8: fatal VF starting between 1 and 3 min 59 s; 9: fatal VF starting <1 min after occlusion.

Hormone analysis

Blood samples for oxytocin and steroid analysis were taken from tails in control, OT, and St groups after anesthesia. Blood samples were centrifuged at 5000 rpm, 4 °C for 5 min in tubes containing EDTA (10% solution, 10 μl/100 μl blood), aprotinin (a protease inhibitor 10 μl/tube) and PMSF (5 μl/tube) and plasma aliquots were frozen at −70 °C until assay.

Oxytocin

Oxytocin was analyzed in extracted plasma using an enzyme-linked immunoassay (ELISA; Phoenix Pharmaceuticals Inc., Burlingame, CA, USA) kit. The sensitivity of the assay was 0.11 pg/ml. The intraassay coefficients of variation were 6.8%.

Corticosterone

Plasma corticosterone concentration as a stress marker was measured using an ELISA (DRG, Marburg, Germany) kit. The sensitivity of assay was 1.63 nmol/l. The intraassay coefficients of variation were 6.4%.

Chemicals

Atosiban, oxytocin acetate salt hydrate, TTC, and sodium pentobarbital were obtained from Sigma–Aldrich and heparin sodium was acquired from Caspian Tamin Pharmaceutical Co. (Tehran, Iran).

Statistical analysis

The hemodynamic parameters were analyzed using repeated measures of analysis of variance (ANOVA) with treatment and stress as grouping factors and time as a repeated factor followed by Tukey's post hoc test. Differences in infarct size, CK-MB, and LDH were evaluated by two-way ANOVA (stress and treatment as factors). When significant interaction was found, Tukey's post hoc test was used for comparison between pair groups. Statistical analysis of hormone levels was determined by one-way ANOVA and unpaired *t*-test.

Arrhythmia scores were analyzed with Kruskal–Wallis test followed by post hoc test Mann–Whitney, 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 $p < 0.05$.

Results

Cardiac function

Cardiac function data observed at the baseline, end of ischemia, and reperfusion in all groups are reported in Table 1. Since HR and LVDP may recover to different degrees, rate pressure product (RPP) was calculated by multiplying heart rate by LVDP and data are presented as a reliable left ventricular function parameter for

Table 1
Cardiac function.

Group	Baseline				End of ischemia			End of reperfusion			
	HR (bpm)	LVDP (mmHg)	RPP (bpm mmHg)	CF (ml/min)	HR (bpm)	LVDP basal value%	RPP basal value%	CF (ml/min)	HR (bpm)	LVDP basal value%	RPP Basal value%	CF (ml/min)
con	288 ± 23	82 ± 5.1	24,063 ± 3572	6 ± 0.6	273 ± 19	59 ± 10	42 ± 8.8	4 ± 0.4	245 ± 15[&]	50 ± 14[&]	46 ± 6.2[&]	3 ± 0.4[&]
St	300 ± 13	90 ± 6.7	27,037 ± 2726	7.4 ± 1.1	233 ± 25	70 ± 6.9	67 ± 6.1	4.2 ± 0.6	230 ± 24[&]	70 ± 9.8[&]	77 ± 3.8[*,&]	3 ± 0.8[&]
ATO + St	266 ± 29	92 ± 6	24,493 ± 2635	6.3 ± 0.6	234 ± 21	70 ± 16	51.6 ± 9.4	2.8 ± 0.6	205 ± 12[&]	69 ± 7.3[&]	48 ± 10[&]	2.8 ± 0.4[&]
OT + St	290 ± 29	83 ± 7.1	24,386 ± 3290	5.8 ± 0.7	230 ± 27	63 ± 11	45 ± 10.4	3.2 ± 0.3	239 ± 24[&]	76 ± 5.4[&]	66 ± 7.9[*,&]	2.6 ± 0.3[&]
OT	302 ± 24	82 ± 8.3	21,865 ± 3030	5.6 ± 0.9	203 ± 25	63.7 ± 7	45.5 ± 9.7	2 ± 1	208 ± 29[&]	84 ± 3.7[&,*]	76 ± 1.5[*,&]	2 ± 0.6[&]
ATO + OT	282 ± 35	70 ± 9.2	18,345 ± 1499	5.7 ± 0.9	245 ± 30	55.6 ± 9	52.4 ± 4.3	3.7 ± 0.7	213 ± 30[&]	38.6 ± 4[&,$]	39 ± 4.2[$,&]	3.6 ± 0.3[&]
ATO	285 ± 27	99 ± 17	27,282 ± 4914	7.3 ± 0.3	264 ± 24	49.7 ± 6	48.8 ± 10	3.6 ± 0.12	239 ± 29[&]	58 ± 10.5[&]	43 ± 13.5[&]	3.5 ± 0.1[&]

ATO, atosiban; CF, coronary flow; con, control; HR, heart rate; LVDP, left ventricular developed pressure; OT, oxytocin; RPP, rate pressure product; St, stress; Data are presented as mean ± SEM.
[&] $p < 0.001$ vs. baseline.
[*] $p < 0.05$ vs. con group.
[$] $p < 0.01$ vs. OT group.

Fig. 2. Myocardial area at risk (AAR/V %) and infarct size (IS/AAR %) in con, St, ATO + St, OT + St, OT, ATO + OT, ATO groups. Data are presented as mean ± SEM. [*] $p < 0.05$ vs. con group. [#] $p < 0.05$ vs. St group. [$] $p < 0.05$ vs. OT group. con, control; St, stress; OT, oxytocin; ATO, atosiban.

the isolated heart. In the baseline period all hearts had a similar RPP, LVDP, HR, and coronary flow (CF).

There were significant differences between RPP under baseline, end of ischemia, and end of reperfusion (repeated measures ANOVA, $F_{(2,62)} = 115.7$, $p < 0.001$). The main effects of stress and treatment were not significant. Results showed a significant interaction between treatment and time on RPP ($F_{(6,62)} = 3.6$, $p < 0.001$). After combining treatment and stress groups, Tukey's post hoc test revealed RPP in St hearts was significantly higher than control group at the end of reperfusion period ($p < 0.05$). Similarly oxytocin administration before stress significantly increased RPP ($p < 0.05$). I.c.v. administration of atosiban prior to stress caused a non-significant decrease in RPP in the ATO + St group compared to the St group. Central pretreatment with oxytocin in the OT group increased RPP in comparison with the control group ($p < 0.05$). Administration of atosiban prior to oxytocin restored RPP to that of the control group in the ATO + OT group ($p < 0.01$). No significant differences were shown between ATO and control groups.

Area at risk and infarct size

No significant treatment by stress interaction and no main effect of treatment and stress were revealed on AAR. Significant main effect was found for treatment on infarct size (two-way ANOVA, $F_{(3,32)} = 21.35$, $p < 0.001$). The main effect of stress was not significant. The results showed a significant interaction between treatment and stress for infarct size ($F_{(2,32)} = 6.73$, $p < 0.05$). For a combination of treatment and stress groups, all pairs of means were compared by Tukey's post hoc test adjusting for multiple comparisons. Stress significantly decreased infarct size in the St group compared to the control group (25.5 ± 2.6 IS/AAR % vs. 41.5 ± 2.8 IS/AAR %, $p < 0.05$). Oxytocin administration before stress significantly decreased the infarct size to 29.8 ± 4 IS/AAR % in the St + OT group from 41.5 ± 2 IS/AAR % in the control group ($p < 0.05$). Central administration of atosiban in the ATO + St group restored infarct size to those of the control group in comparison with the St group (48.3 ± 3.6 IS/AAR % vs. 25.5 ± 2.6 IS/AAR %, $p < 0.001$). Pretreatment with oxytocin prior to stress could not decrease infarct size compared to the St group. Central infusion of oxytocin in the OT group decreased significantly infarct size compared to the control group (22.5 ± 1.9 IS/AAR % vs. 41.5 ± 2.8 IS/AAR %, $p < 0.001$), whereas administration of atosiban prior to oxytocin caused a significant increase in infarct size in the ATO + OT group in comparison with the OT group (54.8 ± 5.8 IS/AAR % vs. 22.5 ± 2.6 IS/AAR %, $p < 0.001$). However, atosiban on its own had no significant effect on infarct size with respect to the control group (Fig. 2).

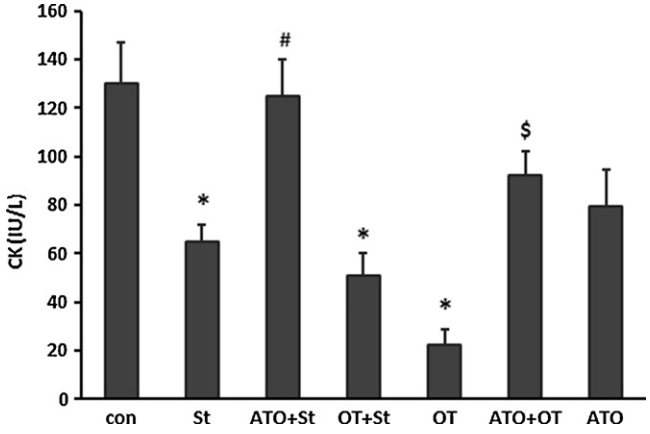

Fig. 3. Level of creatine kinase (CK)-MB in coronary effluent at the end of reperfusion in con, St, ATO + St, OT + St, OT, ATO + OT, ATO groups. Data are presented as mean ± SEM. $^{*}p < 0.05$ vs. con group. $^{\#}p < 0.05$ vs. St group. $^{\$}p < 0.05$ vs. OT group. con, control; St, stress; OT, oxytocin; ATO, atosiban.

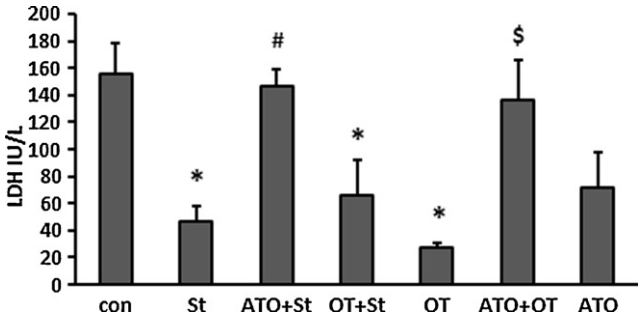

Fig. 4. Level of lactate dehydrogenase (LDH) in coronary effluent at the end of reperfusion in con, St, ATO + St, OT + St, OT, ATO + OT, ATO groups. Data are presented as mean ± SEM. $^{*}p < 0.05$ vs. con group. $^{\#}p < 0.05$ vs. St group. $^{\$}p < 0.05$ vs. OT group. con, control; St, stress; OT, oxytocin; ATO, atosiban.

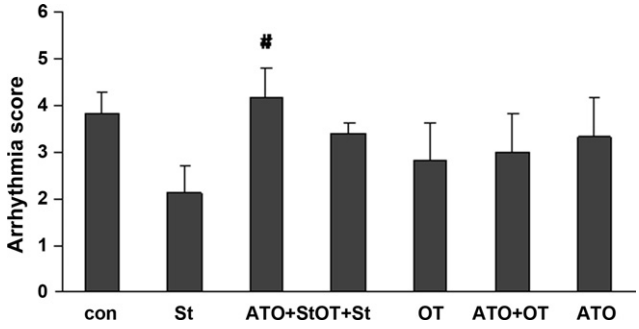

Fig. 5. Distribution of the arrhythmia score during 30 min ischemia in con, St, ATO + St, OT + St, OT, ATO + OT, ATO groups. Data are presented as mean ± SEM. $^{\#}p < 0.05$ vs. St group. con, control; St, stress; OT, oxytocin; ATO, atosiban.

Biochemical analysis

LDH and CK in coronary effluent

A significant main effect was found for treatment on CK-MB and LDH (two-way ANOVA; $F_{(3,30)} = 12.29$, $p < 0.001$ and $F_{(3,32)} = 4.67$, $p < 0.01$ respectively). The main effect of stress was not significant on CK-MB and LDH. The results showed a significant interaction between treatment and stress on CK-MB and LDH ($F_{(2,30)} = 11.38$, $p < 0.001$ and $F_{(2,32)} = 9.34$, $p < 0.001$, respectively). All pairs of means were compared by Tukey's post hoc test adjusting for multiple comparisons. Stress administration prior to ischemia/reperfusion significantly decreased CK and LDH levels in coronary effluent (64.8 ± 7.17 IU/l vs. 130 ± 17.2 IU/l and 47 ± 11.3 IU/l vs. 155.4 ± 22.9 IU/l, respectively, both $p < 0.05$). Similarly, significant differences were found in CK and LDH levels between St + OT and control groups (51 ± 9 IU/l vs. 130 ± 17 IU/l, $p < 0.05$ and 66.2 ± 26.3 IU/l vs. 155.4 ± 22 IU/l, $p < 0.05$, respectively). Central administration of atosiban prior to stress in the ATO + St group significantly increased CK and LDH levels compared to the St group (125 ± 15.3 IU/l vs. 64.8 ± 7.17 IU/l and 146.8 ± 11.8 IU/l vs. 47 ± 11.3 IU/l, respectively, both $p < 0.05$). CK and LDH levels in coronary effluent were markedly declined by central infusion of oxytocin compared to control at the end of reperfusion period (22.2 ± 6.6 IU/l vs. 130 ± 17.2 IU/l, $p < 0.001$ and 27.2 ± 3.6 IU/l vs. 155.4 ± 22.9 IU/l, $p < 0.01$ respectively). However i.c.v. administration of atosiban prior to oxytocin increased CK and LDH compared to the OT group (92.2 ± 10 IU/l vs. 22.25 ± 6.6 IU/l, $p < 0.01$ and 136.86 ± 29.6 IU/l vs. 27.2 ± 3.6 IU/l, $p < 0.05$, respectively, $p < 0.05$). Intraventricular infusion of oxytocin prior to stress in the OT + St group, had no significant effect on CK and LDH levels compared to the St group. Administration of atosiban alone prior to ischemia/reperfusion had no significant effect on CK and LDH in comparison with control (Figs. 3 and 4).

Ventricular arrhythmias during ischemia

Severity of arrhythmias

Administration of stress prior to ischemia did not significantly decrease ischemia-induced ventricular arrhythmias severity with respect to control group (2.1 ± 0.55 arrhythmia score vs. 3.85 ± 0.45 arrhythmia score, $p < 0.05$). But administration of atosiban prior to stress in the ATO + St group intensified severity of arrhythmia compared to the St group (4.1 ± 0.65 arrhythmia score vs. 2.1 ± 0.55 arrhythmia score, $p < 0.05$). Arrhythmia severity was similar in hearts of the St and OT + St groups. Central administration of oxytocin alone had no significant effect on arrhythmia severity (Fig. 5).

Incidence of VT and VF

In the control group, the incidence of VT and VF occurred in 100% and 14.3% of hearts, respectively. No significant differences were observed in VF incidence among groups. Administration of stress prior to ischemia significantly decreased incidence of VT with respect to the control group. Central administration of atosiban prior to stress in the ATO + St group significantly increased VT incidence compared to the St group ($p < 0.05$). The incidence of VT markedly declined by central infusion of oxytocin compared to controls. However i.c.v. administration of atosiban prior to oxytocin increased VT incidence compared to the OT group ($p < 0.05$) (Fig. 6).

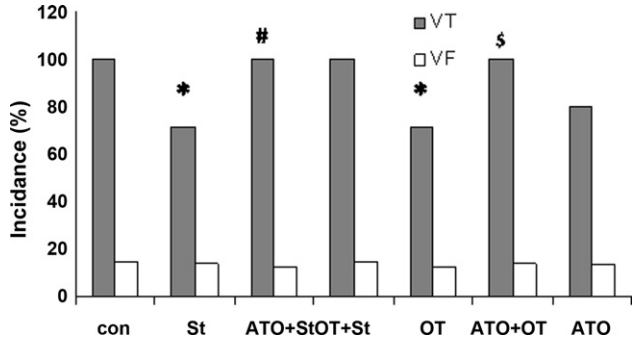

Fig. 6. Incidence of ventricular tachycardia (VT) and ventricular fibrillation (VF) during 30 min ischemia in con, St, ATO + St, OT + St, OT, ATO + OT, ATO groups. Data are presented as mean ± SEM. $^{*}p < 0.05$ vs. con group. $^{\#}p < 0.05$ vs. St group. $^{\$}p < 0.05$ vs. OT group. con, control; St, stress; OT, oxytocin; ATO, atosiban.

Plasma hormone level

The plasma concentrations of oxytocin and corticosterone were 7.38 ± 0.6 pg/ml and 26.2 ± 5.07 nmol/l, respectively in the control group. However, following induction of stress these concentrations significantly rose to mean concentrations of 17.9 ± 4.2 pg/ml and 41.7 ± 4.4 nmol/l, respectively. Administration of oxytocin in the OT group did not change the plasma oxytocin level in comparison to the control group (one way ANOVA, $p < 0.05$).

Discussion

This study provides the first demonstration of the implication of central oxytocin released and its receptors in stress-induced cardioprotection. We observed that stress significantly decreased infarct size, incidence of VT, level of CK and LDH, and increased RPP in isolated rat hearts subjected to an ischemia–reperfusion sequence. Stress-induced myocardial tolerance to ischemia was abolished by i.c.v. administration of atosiban (as an oxytocin receptor antagonist) prior to stress exposure. To our knowledge this is the first report of the cardioprotective effect of i.c.v. infusion of oxytocin against IR injury in the heart which was inhibited by the administration of atosiban (i.c.v.).

It is well known that acute cardiovascular events (acute coronary syndrome and stroke) can be triggered by abrupt emotional or physical stressors [25] and stress can also be harmful to the cardiovascular system [26]. Many clinical studies reported that exposure to stress correlates with increased morbidity and mortality from cardiovascular diseases, including myocardial ischemia [27,28]. Acute stress accelerates the HR, cardiac contractility, and total peripheral resistance [29] and consequently, cardiac work and oxygen consumption may markedly increase [30,31]. Forced exercise can induce maladjusted changes in both brain and heart tissues [32,33]. Mancardi et al. observed a worsening of IR outcomes in the heart of rats forced to run experiment [34]. Scheuer and Mifflin also showed that chronic stress increased the size of infarction [35]. These observations conflict with other studies showing heat stress significantly reduced infarct size in the isolated rat heart subjected to an IR sequence [36,37]. Acute exercise training [38,39] and repeated physiologic stress provided myocardial protection against IR injury [40]. Moreover there is evidence for cold-restraint stress role in cardioprotection [41].

In our study, an acute episode of stress experienced just before IR could provide myocardial protection against IR injury and decrease infarct size as the hardest end point of ischemic heart injury. The major effects of cardioprotection are reduction in infarct size (anti necrotic effect) [42], reduction in number and severity of cardiac arrhythmias (anti arrhythmic effect) [43,44], and improvement in contractile performance (protection against contractile dysfunction) [45]. Stress pretreatment improved contractile function, and reduced incidence of VT and biochemical parameters in the same direction as the infarct size in our experiment. Elevated levels of CK have been regarded as a specific biochemical marker of myocyte necrosis [46] and LDH level plays an important role in systemic tissue damage [47].

It has been documented that stress evokes a few protective responses which can prevent the development of IR injury [35]. The stress response is probably due to interactions between the neuroendocrine system, the sympathetic nervous system, and the target organs, which result in the release of specific hormones [48]. Previous studies show that a 10-min forced swimming session triggers the release of oxytocin within the hypothalamic SON and the PVN [15,19], which parallels oxytocin secretion into the blood [15,49]. Our study showed that oxytocin plasma concentration was increased by swim stress, confirming the results of previous studies. Hence in the present experiment, central infusion of atosiban inhibited the protective effect of stress in the ATO + St group and abolished the effects of stress on infarct size, RPP, incidence of VT and release of CK and LDH. Since atosiban does not cross the blood–brain barrier [50], the inhibitory effects of atosiban may be mediated via brain oxytocin receptors. These findings seem to exclude the possibility that centrally released endogenous oxytocin in response to stress could protect and prevent worsening or development of IR injury by activation of central oxytocin receptors. Oxytocin is considered to be an endogenous stress-relieving compound [51]. Wsol et al. showed that oxytocin buffers the cardiovascular responses to stress and with central blockade of oxytocin receptors indicated that centrally released endogenous oxytocin significantly attenuates the intensity of the cardiovascular responses to acute stress in intact rats [7]. It is also shown that infusion of oxytocin into the brain prior to stress did not change the cardiovascular responses to stress [7]. In addition, our previous study assessing the role of peripherally released oxytocin in stress provided the same result [52]. The role of oxytocin in the central regulation of the cardiovascular system at rest was previously investigated by many authors but the results were inconclusive [53–56]. In some studies intraventricular or intracisternal administration of oxytocin elicited pressor or tachycardiac effects [55,57], whereas in others i.c.v. infusion of oxytocin for 5 days turned out to be hypotensive and reacted with a larger elevation of blood pressure and heart rate in response to unexpected and sudden noise [58]. It has also been found that oxytocin reduces acceleration of the heart rate during exercise [56].

Anatomical studies of oxytocin pathways in the brain have revealed extensive innervations of the brain stem structures regulating the cardiovascular, behavioral, and neuroendocrine responses to stress by oxytocin fibers projecting from the PVN [10], and there is a population of PVN-spinal oxytocin neurons that excite cardiac sympathetic preganglionic neurons controlling heart rate [59]. Moreover, most recently it has been documented that peripheral administration of oxytocin induces a cardioprotective and preconditioning effect on IR injury in both isolated rat heart [42], and anesthetized rats [13]. However thus far, the role of central administration of oxytocin in IR injury has not been investigated.

In our study, central infusion of exogenous oxytocin increased recovery of LVDP and RPP at the end of reperfusion and decreased infarct size, the level of CK and LDH in comparison with the control group, without any changes in oxytocin plasma concentration. Central administration of an oxytocin receptor antagonist that does not cross the blood–brain barrier [50] eliminated the direct stimulatory influence of oxytocin on brain receptor. This implies that the cardioprotective effect of stress and i.c.v. infusion of oxytocin against IR injury may be transmitted in part by the actions of oxytocin in the brain, although oxytocin probably also has a direct cardioprotective effect. Therefore it may be deduced that oxytocin has a preconditioning effect via central action. Interestingly in the present experiment, administration of oxytocin prior to stress did not increase the protection of stress. There are two possible explanations for these findings: first, swimming could stimulate release of oxytocin in multiple ways and the release could be sufficient enough to saturate oxytocin receptors and second, since we have previously reported that oxytocin has a biphasic dose-dependent effect against IR injury [13], the combination of exogenous oxytocin and oxytocin released in response to stress may provide higher doses which show less activity.

In the present study, our model of stress could not cause a significant attenuation in severity of arrhythmias showing that the changes in infarct size do not correlate with the anti-arrhythmic effects of stress. In this regard some studies showed that preconditioning reduces infarct size, but accelerates time to ventricular fibrillation in ischemic heart [60–62]. Therefore, it seems that the extent of myocardial infarction may not directly affect arrhythmia.

Blockade of oxytocin receptor significantly offsets the effects of stress on ventricular arrhythmias. This observation implies beneficial effects of endogenous oxytocin on arrhythmia in stress condition. In this line some studies showed that adaptation to short-term non-damaging stress effect largely limits or prevents cardiac arrhythmias in acute ischemia and reperfusion [63]. A large number of studies demonstrated that mild stress resulted in protection against exposure to subsequent more severe stress [64,65] representing cardiac preconditioning. The present study showed that administration of stress prior to ischemia–reperfusion may have a preconditioning effect.

In conclusion these findings suggest that i.c.v. infusion of exogenous oxytocin and oxytocin released into the brain during stress could induce a preconditioning effect in ischemic-reperfused rat heart via brain receptors.

Acknowledgment

This study was supported by a grant from the Tehran University of Medical Science.

References

[1] Szczepanska-Sadowska E. Role of neuropeptides in central control of cardiovascular responses to stress. J Physiol Pharmacol 2008;59:61–89.
[2] Kishi T. Heart failure as an autonomic nervous system dysfunction. J Cardiol 2012;59:117–22.
[3] Kuwahata S, Miyata M, Fujita S, Kubozono T, Shinsato T, Ikeda Y, Hamasaki S, Kuwaki T, Tei C. Improvement of autonomic nervous activity by Waon therapy in patients with chronic heart failure. J Cardiol 2010;57:100–6.
[4] Kombian SB, Mouginot D, Pittman QJ. Dendritically released peptides act as retrograde modulators of afferent excitation in the supraoptic nucleus in vitro. Neuron 1997;19:903–12.
[5] Neumann ID, Wigger A, Torner L, Holsboer F, Landgraf R. Brain oxytocin inhibits basal and stress-induced activity of the hypothalamo-pituitary-adrenal axis in male and female rats: partial action within the paraventricular nucleus. J Neuroendocrinol 2000;12:235–43.
[6] Martins AS, Crescenzi A, Stern JE, Bordin S, Michelini LC. Hypertension and exercise training differentially affect oxytocin and oxytocin receptor expression in the brain. Hypertension 2005;46:1004–9.
[7] Wsol A, Cudnoch-Jedrzejewska A, Szczepanska-Sadowska E, Kowalewski S, Puchalska L. Oxytocin in the cardiovascular responses to stress. J Physiol Pharmacol 2008;59:123–7.
[8] Gibbs DM. Oxytocin inhibits ACTH and peripheral catecholamine secretion in the urethane-anesthetized rat. Regul Pept 1986;14:125–32.
[9] Gutkowska J, Jankowski M, Mukaddam-Daher S, McCann SM. Oxytocin is a cardiovascular hormone. Braz J Med Biol Res 2000;33:633–725.
[10] Sawchenko PE, Swanson LW. Immunohistochemical identification of neurons in the paraventricular nucleus of the hypothalamus that project to the medulla or to the spinal cord in the rat. J Comp Neurol 1982;205:260–72.
[11] Sofroniew MVSU. Evidence for direct projections from oxytocin and vasopressin neurons in the hypothalamic paraventricular nucleus in the medulla oblongata: immunohistochemical visualization of both the horseradish peroxidase transported and peptide produced by the same neurons. Neurosci Lett 1981;22:211–7.
[12] Petersson M, Lundeburg AP, Uvnäs-Moberg TK. Oxytocin causes a long time decrease of blood pressure in female and male rats. Physiol Behav 1996;60:1311–5.
[13] Houshmand F, Faghihi M, Zahediasl S. Biphasic protective effect of oxytocin on cardiac ischemia/reperfusion injury in anaesthetized rats. Peptides 2009;30:2301–8.
[14] Alizadeh AM, Faghihi M, Sadeghipour HR, Mohammadghasemi F, Imani A, Houshmand F, Khori V. Oxytocin protects rat heart against ischemia–reperfusion injury via pathway involving mitochondrial ATP-dependent potassium channel. Peptides 2010;31:1341–5.
[15] Wotjak CT, Ganster J, Kohl G, Holsboer F, Landgraf R, Engelmann M. Dissociated central and peripheral release of vasopressin, but not oxytocin, in response to repeated swim stress: new insights into the secretory capacities of peptidergic neurons. Neuroscience 1998;85:1209–22.
[16] Neumann ID. Stimuli and consequences of dendritic release of oxytocin within the brain. Biochem Soc Trans 2007;35:1252–7.
[17] Lang RE, Heil JW, Ganten D, Hermann K, Unger T, Rascher W. Oxytocin unlike vasopressin is a stress hormone in the rat. Neuroendocrinology 1983;37:314–6.
[18] Jorgensen H, Knigge U, Kjaer A, Warberg J. Serotonergic involvement in stress-induced vasopressin and oxytocin secretion. Eur J Endocrinol 2002;147:815–24.
[19] Wotjak CT, Naruo T, Muraoka S, Simchen R, Landgraf R, Engelmann M. Forced swimming stimulates the expression of vasopressin and oxytocin in magnocellular neurons of the rat hypothalamic paraventricular nucleus. Eur J Neurosci 2001;13:2273–81.
[20] Naderi R, Imani A, Faghihi M. Phenylephrine produces late pharmacological preconditioning in the isolated rat heart. Eur J Pharmacol 2010;627:203–8.
[21] Naderi R, Imani A, Faghihi M, Moghimian M. Phenylephrine induces early and late cardioprotection through mitochondrial permeability transition pore in the isolated rat heart. J Surg Res 2010;164:37–42.
[22] Imani A, Faghihi M, Keshavarz M, Karimian SM, Niaraki SS. Effect of different doses of noradrenaline against ischemia-induced ventricular arrhythmias in rat heart in vivo. Indian Pacing Electrophysiol J 2009;9:35–44.
[23] Walker MJCM, Hearse DJ, Campbell RW, Janse MJ, Yellon DM, Cobbe SM, Coker SJ, Harness JB, Harron DW. The Lambeth conventions: guidelines for the study of arrhythmias in ischaemia infarction, and reperfusion. Cardiovasc Res 1988;22:447–55.
[24] Sarraf GBT, Walker MJ. Tedisamil and lidocaine enhance each other's antiarrhythmic activity against ischemia-induced arrhythmias in rats. Br J Pharmacol 2003;101:439–45.
[25] Rozanski A, Blumenthal JA, Kaplan J. Impact of psychological factors on the pathogenesis of cardiovascular disease and implications for therapy. Circulation 1999;99:2192–217.
[26] Ramachandruni S, Handberg E, Sheps DS. Acute and chronic psychological stress in coronary disease. Curr Opin Cardiol 2004;19:494–9.
[27] Krantz DS, Kop WJ, Santiago HT, Gottdiener JS. Mental stress as a trigger of myocardial ischemia and infarction. Cardiol Clin 1996;14:271–87.
[28] Tennant CC, Palmer KJ, Langeluddecke PM, Jones MP, Nelson G. Life event stress and myocardial reinfarction: a prospective study. Eur Heart J 1994;15:472–8.
[29] Zhang WHB, Leenen FH. Brain renin–angiotensin system and sympathetic hyperactivity in rats after myocardial infarction. Am J Physiol 1999;276:1608–15.
[30] Clarke SP, Frasure-Smith N, Lesperance F, Bourassa MG. Psychosocial factors as predictors of functional status at 1 year in patients with left ventricular dysfunction. Res Nurs Health 2000;23:290–300.
[31] Kario K, McEwen BS, Pickering TG. Disasters and the heart: a review of the effects of earthquake-induced stress on cardiovascular disease. Hypertens Res 2003;26:355–67.
[32] Koller A, Mertelseder S, Moser H. Is exercise-induced myocardial injury self-abating? Med Sci Sports Exerc 2001;33:850–1.
[33] Scopel D, Fochesatto C, Cimarosti H, Rabbo M, Bello-Klein A, Salbego C, Netto CA, Siqueira IR. Exercise intensity influences cell injury in rat hippocampal slices exposed to oxygen and glucose deprivation. Brain Res Bull 2006;71:155–9.
[34] Mancardi D, Tullio F, Crisafulli A, Rastaldo R, Folino A, Penna C, Pagliaro P. Omega 3 has a beneficial effect on ischemia/reperfusion injury, but cannot reverse the effect of stressful forced exercise. Nutr Metab Cardiovasc Dis 2009;19:20–6.
[35] Scheuer DA, Mifflin SW. Repeated intermittent stress exacerbates myocardial ischemia–reperfusion injury. Am J Physiol 1998;274:470–5.
[36] Joyeux M, Godin-Ribuot D, Patel A, Demenge P, Yellon DM, Ribuot C. Infarct size-reducing effect of heat stress and alpha 1 adrenoceptors in rats. Br J Pharmacol 1998;125:645–50.
[37] Arnaud C, Laubriet A, Joyeux M, Godin-Ribuot D, Rochette L, Demenge P, Ribuot C. Role of nitric oxide synthases in the infarct size-reducing effect conferred by heat stress in isolated rat hearts. Br J Pharmacol 2001;132:1845–51.
[38] Locke M, Tanguay RM, Klabunde RE, Ianuzzo CD. Enhanced postischemic myocardial recovery following exercise induction of HSP 72. Am J Physiol 1995;269:320–5.
[39] Taylor RP, Harris MB, Starnes JW. Acute exercise can improve cardioprotection without increasing heat shock protein content. Am J Physiol 1999;276:1098–102.
[40] Hoshida S, Yamashita N, Otsu K, Hori M. Repeated physiologic stress provide persistent cardioprotection against ischemia–reperfusion injury in rats. J Am Coll Cardiol 2002;40:826–31.
[41] Wu S, Wong MC, Chen M, Cho CH, Wong TM. Role of opioid receptors in cardioprotection of cold-restraint stress and morphine. J Biomed Sci 2004;11:726–31.
[42] Ondrejcakova M, Ravingerova T, Bakos J, Pancza D, Jezova D. Oxytocin exerts protective effects on in vitro myocardial injury induced by ischemia and reperfusion. Can J Physiol Pharmacol 2009;87:137–42.
[43] Banerjee A, Locke-Winter C, Rogers KB, Mitchell MB, Brew EC, Cairns CB, Bensard DD, Harken AH. Preconditioning against myocardial dysfunction after ischemia and reperfusion by an alpha 1-adrenergic mechanism. Circ Res 1993;73:656–70.
[44] Headrick JP. Ischemic preconditioning: bioenergetic and metabolic changes and the role of endogenous adenosine. J Mol Cell Cardiol 1996;28:1227–40.
[45] Tsuchida A, Liu Y, Liu GS, Cohen MV, Downey JM. alpha 1-Adrenergic agonists precondition rabbit ischemic myocardium independent of adenosine by direct activation of protein kinase C. Circ Res 1994;75:576–85.
[46] Yilmaz A, Yalta K, Turgut OO, Yilmaz MB, Ozyol A, Kendirlioglu O, Karadas F, Tandogan I. Clinical importance of elevated CK-MB and troponin I levels in congestive heart failure. Adv Ther 2006;23:1060–7.
[47] Devi R, Banerjee SK, Sood S, Dinda AK, Maulik SK. Extract from *Clerodendron colebrookianum* Walp protects rat heart against oxidative stress induced by ischemic-reperfusion injury (IRI). Life Sci 2005;77:2999–3009.
[48] Bohus B, Benus RF, Fokkema DS, Koolhaas JM, Nyakas C, van Oortmerssen GA, Prins AJ, de Ruiter AJ, Scheurink AJ, Steffens AB. Neuroendocrine states and behavioral and physiological stress responses. Prog Brain Res 1987;72:57–70.
[49] Wigger A, Neumann ID. Endogenous opioid regulation of stress-induced oxytocin release within the hypothalamic paraventricular nucleus is reversed in late pregnancy: a microdialysis study. Neuroscience 2002;112:121–9.

[50] McKee DT, Poletini MO, Bertram R, Freeman ME. Oxytocin action at the lactotroph is required for prolactin surges in cervically stimulated ovariectomized rats. Endocrinology 2007;148:4649–57.
[51] Neumann ID. Brain oxytocin: a key regulator of emotional and social behaviours in both females and males. J Neuroendocrinol 2008;20:858–65.
[52] Moghimian M, Faghihi M, Karimian SM, Imani A. The effect of acute stress exposure on ischemia and reperfusion injury in rat heart: role of oxytocin. Stress 2012;15:385–92.
[53] Braga DC, Mori E, Higa KT, Morris M, Michelini LC. Central oxytocin modulates exercise-induced tachycardia. Am J Physiol Regul Integr Comp Physiol 2000;278:1474–82.
[54] Charpak S, Armstrong WE, Muhlethaler M, Dreifuss JJ. Stimulatory action of oxytocin on neurons of the dorsal motor nucleus of the vagus nerve. Brain Res 1984;300:83–9.
[55] Feuerstein GZR, Faden AI. Central cardiovascular effects of vasotocin, oxytocin and vasopressin in conscious rats. J Pharmacol Exp Ther 1984;228: 348–53.
[56] Michelini LC. Oxytocin in the NTS. A new modulator of cardiovascular control during exercise. Ann N Y Acad Sci 2001;940:206–20.
[57] Tran LD, Montastruc JL, Montastruc P. Effects of lysine-vasopressin and oxytocin on central cardiovascular control. Br J Pharmacol 1982;77:69–73.
[58] Petersson M, Uvnas-Moberg K. Effects of an acute stressor on blood pressure and heart rate in rats pretreated with intracerebroventricular oxytocin injections. Psychoneuroendocrinology 2007;32:959–65.
[59] Yang Z, Han D, Coote JH. Cardiac sympatho-excitatory action of PVN-spinal oxytocin neurons. Auton Neurosci 2009;147:80–5.
[60] Ovize M, Aupetit JF, Rioufol G, Loufoua J, Andre-Fouet X, Minaire Y, Faucon G. Preconditioning reduces infarct size but accelerates time to ventricular fibrillation in ischemic pig heart. Am J Physiol 1995;269:72–9.
[61] Murry CEJR, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. Circulation 1986;74:1124–36.
[62] Taggart PYD. Preconditioning and arrhythmias. Circulation 2002;10:2999–3001.
[63] Malyshev FZ, Mal Y. Adaptation to stress increases the heart resistance to ischemic and reperfusion arrhythmias. J Mol Cell Cardiol 1989;21:299–303.
[64] Parsell DALS. The function of heat-shock proteins in stress tolerance: degradation and reactivation of damaged proteins. Annu Rev Genet 1993;27:437–96.
[65] Lindquist SCE. The heat shock proteins. Annu Rev Genet 1988;22:631–77.