

Evaluation of Chronic Physical and Psychological Stress Induction on Cardiac Ischemia / Reperfusion Injuries in Isolated Male Rat Heart: The Role of Sympathetic Nervous System

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Abstract- Exposure to stress leads to physiological changes called “stress response” which are the result of the changes in the adrenomedullary hormone system, hypothalamus-pituitary-adrenal (HPA) and sympathetic nervous system (SNS) activity. In the present study, the effects of chronic physical and psychological stress and also the role of sympathetic system effects in stress on ischemia/reperfusion (I/R) injuries have been studied in isolated rat heart. Rat heart was isolated and subjected to 30 min regional ischemia and 120 min reperfusion. The daily stress was induced for one week prior to I/R induction. Sympathectomy was done chemically by injection of hydroxyl-dopamine prior to stress induction. There were no significant changes in heart rate and Coronary Flow between groups. Left ventricular developed pressure (LVDP) and rate product pressure (RPP) in both physical and psychological stress groups decreased significantly compared to those in control group ($P<0.05$), but there was no significant difference between physical and psychological stress groups. Infarct size significantly increased in both physical and psychological stress groups and control group ($P<0.05$). Sympathectomy before induction of stress led to the elimination of the deleterious effects of stress as compared with stress groups ($P<0.05$). These results show that induction of chronic physical and psychological stress prior to ischemia/reperfusion causes enhancement of myocardial injuries and it seems that increased sympathetic activity in response to stress is responsible for these adverse effects of stress on ischemic/reperfused heart.

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Introduction

Stress is a series of reactions that can be created in response to physical, psychological or any other stimuli that cause hemostasis disturbance (1). Stress symptoms commonly include a state of alarm and adrenaline production, short-term resistance as a coping mechanism, and exhaustion. Irritability, muscular tension, inability to concentrate and a variety of physiological reactions such as headache, elevated heart rate, inability to relax, feeling lonely, isolated or depressed, aches and pains, diarrhea or constipation,

nausea, dizziness, chest pain, and increased alcohol and nicotine consumption are also mentioned in the literature (2-4). It has been reported that general stress response of rodent, as subjected to physiological, psychological and environmental stressors, is activation of sympathoadrenomedullary (SAM) system and hypothalamic-pituitary-adrenal (HPA) axis and it is suggested that these coordinated responses of the two physiological axes improve individual's stress resistance and result in release of glucocorticoids and catecholamine. Exposure to stress causes physiological changes called “stress response” which involve changes

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in both HPA and sympathetic nervous system (SNS) activity and also changes such as shift of energy to brain and muscles, an increase in velocity of brain perfusion, an increase in glucose uptake, enhancement of cardiac and respiratory output and a decrease in appetite (5, 6). The autonomic nervous system provides a rapid response to stress commonly known as the fight-or-flight response, engaging the sympathetic nervous system and withdrawing the parasympathetic nervous system (7). As a result of the stimulation of HPA, blood glucocorticoids (cortisol in humans and corticosterone in rodents), other neuroendocrine, autonomic and behavioral responses increase and become coordinated and in addition some adaptive changes in both energy and behavior to stress occur (8,9). Chronic stress plays an important role in the etiology of depressive disorders (10). One of the important physiological pathways of the stress response is SAM system, and several paradoxical phenomena have been observed. For example, catecholamine deficiency in the brain is thought to be associated with depression, whereas excessive catecholamine contributes to stress-induced excitotoxicity (11,12). The notion that psychological stress can lead to the development of cardiovascular and metabolic diseases is entrenched in the public psyche, but the scientific evidence underpinning this association has been slow to accumulate (13). Previous studies have reported stress influence on the clinical diseases (14-16) but determining and comparing the effect of chronic physical and psychological stress on the heart ischemia/reperfusion injuries and the underlying mechanisms are largely unknown. In the present study, the effects of both chronic physical and chronic psychological stress in presence or absence of sympathetic nervous system in ischemia/reperfusion injury in isolated male rat heart was evaluated.

Materials and Methods

Animals

A total of 56 Male Wistar rats (200–250 g) were obtained from Tehran University of Medical Sciences animal house and were housed in an air-conditioned colony room in 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 thiopental (60 mg/kg, 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.

Induction of physical and psychological stress

The communication box (stress box) was used for induction of physical and emotional (psychological) stress paradigms in this study. It consists of 9 compartments (16 × 16 cm) separated by transparent plastic boards. The boards have a few pores to allow animals to receive visual, auditory and olfactory sensation cues from the neighboring animal but prevent them from physical contact. Each compartment was equipped with a grid floor made up of 5 mm diameter stainless steel rods placed 1.3 cm apart each other (17). An electric generator (current of 1 mA) (BorjSanat Co. Iran, sensitivity 0.1-10 mA) was connected to the grid floor to generate an electric foot shock for 10 seconds with an interval of 50 seconds. Four compartments of the grid floor were insulated by plastic plates to prevent electric foot shock and served as non-foot-shock compartments for the emotional stress rats.

Chemical sympathectomy

Male Wistar rats, weighing about 200-250 g, received a single subcutaneous injection of 100 mg/kg body weight of hydroxydopamine (6-OHDA) (Sigma-Aldrich, USA) in 0.9% NaCl with 0.1% ascorbic acid (Daroupakhsh Co. Iran). The solution was prepared immediately before injection and in a light protected condition (18).

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 (19).

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). A surgical needle (6-0 silk suture) was passed under the origin of the left anterior descending coronary artery (LAD), 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 were 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.

Chronic physical and psychological and I/R

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 ($HR \times LVDP$).

Experimental protocol

Rats were randomly divided into seven groups (8 in each group) and received physical and psychological stress before anesthesia and isolated hearts were subjected to 30 min ischemia and 120 min reperfusion. Study groups included; 1: Ischemia-reperfusion control (IR), rats Put in the stress box for 1 hour each day without receiving any form of mentioned stress for 7 days and 24 hours after the end of this period were subjected to isolated heart surgery. 2: chronic physical

stress, Animals were kept in the stress box. They were received a 10-second electrical shock and 50-second rest for 1 hour on a daily basis for 1 week and 24 hours after the end of this period they were subjected to isolated heart surgery. 3: chronic Psychological stress, Animals were put in the stress box for 1 hour each day and exposed to psychological shock for 1 week and 24 hours after end of this period were subjected to isolated heart surgery. 4: sympathectomy. 5: chemical sympathectomy + chronic physical stress. 6: chemical sympathectomy + chronic psychological stress. These three groups was similar to 1, 2, and 3 groups respectively, but animals in these groups received 6-OHDA one day prior to stress induction periods. 7: vehicle, Animal received solvent of 6-OHDA (0.9% NaCl with 0.1% ascorbic acid) one day prior to stress induction period and were subjected as same as a group of 4 (Figure 1).

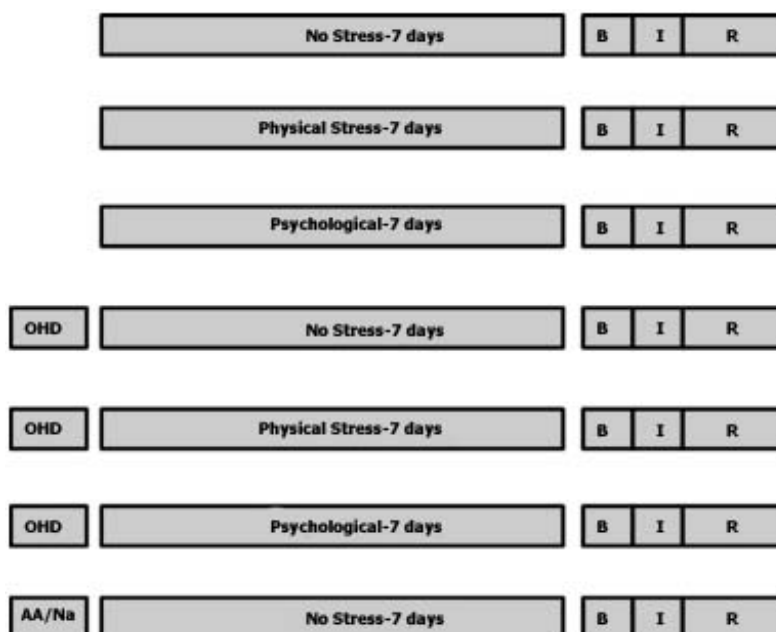


Figure 1. Schematic illustration of experimental groups (n=7)

B: baseline, I: ischemia, R: reperfusion, OHD: hydroxydopamine, AA/Na: ascorbic acid/NaCl

Infarct size measurement

After completion of the reperfusion period, the left coronary artery was re-occluded, and Evans blue dye was infused via the aorta to differentiate the ischemic zone (the 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 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 (Ver. 7.0, Adobe System, San Jose, CA, USA). AAR was expressed as a percentage of left ventricular size for each heart, and the infarct size was expressed as a percentage of AAR (19,20).

Corticosterone

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

Systolic blood pressure

Animal's systolic pressure was measured by tail-cuff and PowerLab apparatus (ADInstruments Co. Australia) before and 24 hours after of sympathectomy in non-invasive blood pressure (NIBP) method (21).

Chemicals

6-OHDA and TTC were obtained from Sigma–Aldrich, sodium thiopental was obtained from Abachem Ltd Co. (England), sodium heparin, and ascorbic acid were acquired from Daroupakhsh Pharmaceutical Co. (Tehran, Iran).

Statistical analysis

Data are expressed as means \pm S.E.M. Statistical comparison of means between groups was made by one-way ANOVA and a subsequent Tukey test for infarcted size ratio and hemodynamic parameters. Within each group, hemodynamic parameters analysis was made by using repeated measurement ANOVA. Significant differences were determined as $P < 0.05$.

Results

Heart rate and coronary flow

Table 1 shows heart rate (HR) and coronary flow (CF) in different groups. Statistical analysis showed that HR and CF were significantly reduced at the end of both of ischemia and reperfusion periods as compared to its baseline in each group ($P < 0.05$). Inter groups comparison showed no significant changes that mean different interventions could not improve or attenuated heart rate and coronary flow in study groups.

Table 1. The amount of HR and CF at the end of the baseline, ischemia, and reperfusion times

Groups	End of Baseline		End of Ischemia		End of Reperfusion	
	HR (bpm)	CF (ml/min)	HR (bpm)	CF (ml/min)	HR (bpm)	CF (ml/min)
IR	306 \pm 24	9 \pm 0/65	203 \pm 25	6 \pm 0/8 [#]	208 \pm 16 [#]	4 \pm 0/8 [#]
VEHICLE	301 \pm 13	10 \pm 0/6	210 \pm 23	4 \pm 1 [#]	215 \pm 24 [#]	3 \pm 0/5 [#]
SYM	290 \pm 11	8 \pm 1/2	204 \pm 15	4/5 \pm 0/4 [#]	202 \pm 19/3 [#]	5 \pm 0/15 [#]
PHY	282 \pm 20	9 \pm 0/7	210 \pm 33	5 \pm 1 [#]	203 \pm 30 [#]	4 \pm 1/05 [#]
PSC	279 \pm 23	8 \pm 0/6	201 \pm 19	4/5 \pm 1/3 [#]	200 \pm 15 [#]	3 \pm 0/6 [#]
PHY+SYM	296 \pm 19	8 \pm 1	198 \pm 21	4 \pm 0/9 [#]	205 \pm 13 [#]	4 \pm 1 [#]
PSC+SYM	289 \pm 19	11 \pm 1/5	208 \pm 10	5/5 \pm 1 [#]	219 \pm 8 [#]	5 \pm 0/65 [#]

HR: heart rate, CF: coronary flow, bpm: beat per minute, I/R: ischemia/reperfusion, SYM: control of sympathectomy, PHY: physical stress, PSC: psychological stress, PHY+SYM: Sympathectomy+physical stress, PSC+SYM: Sympathectomy+psychological stress. Data are presented as mean \pm S.E.M. # $P < 0.05$ compared to their baselines. n=8 per group

Hemodynamic parameters

Left ventricular developed pressure (LVDP) and rate product pressure (RPP) was decreased in all groups at the end of reperfusion compared to their baseline ($P < 0.05$). Inter groups comparison showed that amount of LVDP and RPP in both physical and psychological stress significantly decrease compared to IR group ($P < 0.05$). LVDP in both sympathectomy with physical and sympathectomy with psychological stress was significantly increased in comparison to physical and psychological stress groups respectively ($P < 0.05$) but they did not show any difference compared to IR group.

RPP in both sympathectomy with physical and sympathectomy with psychological stress was non-significantly increased in comparison to physical and psychological stress groups respectively (Figure 2, 3).

Infarct size and area at risk

There were no significant differences in the ratio of the area at risk to the total left ventricular area between the hearts in all experimental groups. Findings indicated that coronary arteries (LAD) in all of the hearts were ligated identically (Figure 4).

Chronic physical and psychological and I/R

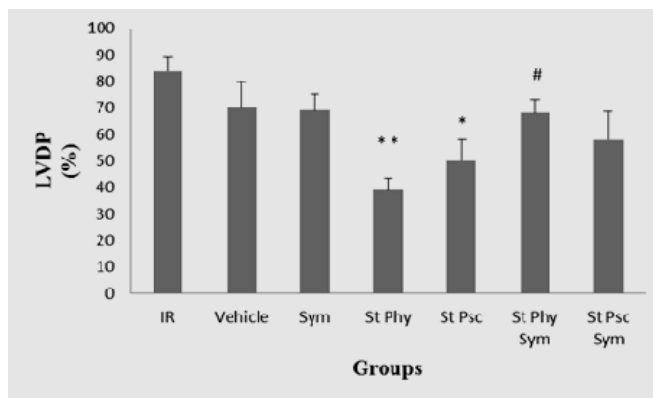


Figure 2. The ratio of Left Ventricular Developed Pressure (LVDP) at the end of reperfusion to their basal value in different groups (n=8)
 I/R: ischemia/reperfusion, SYM: control of sympathectomy, PHY: physical stress, PSC: psychological stress, PHY+SYM: Sympathectomy+physical stress, PSC+SYM: Sympathectomy+ psychological stress. Data are presented as mean±S.E.M.
 ** $P < 0.01$ and * $P < 0.05$ compared to IR group. # $P < 0.05$ compared to physical stress group

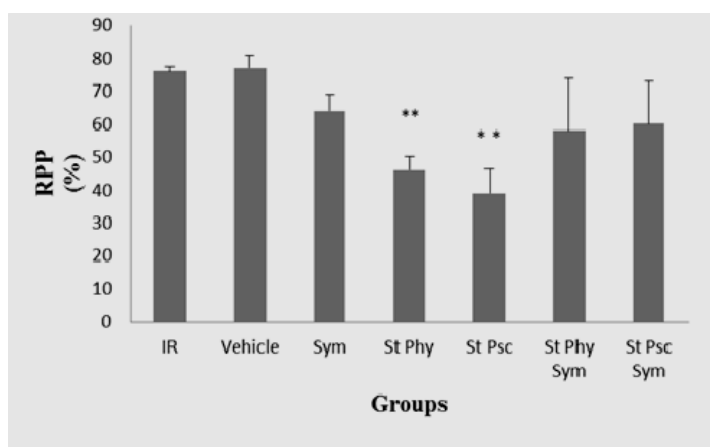


Figure 3. The ratio of Rate Product Pressure (RPP) at the end of reperfusion to their basal value in different groups (n=8)
 I/R: ischemia/reperfusion, SYM: control of sympathectomy, PHY: physical stress, PSC: psychological stress, PHY+SYM: Sympathectomy+physical stress, PSC+SYM: Sympathectomy+ psychological stress. Data are presented as mean ± S.E.M.
 * $P < 0.05$ and ** $P < 0.01$ compared to IR group

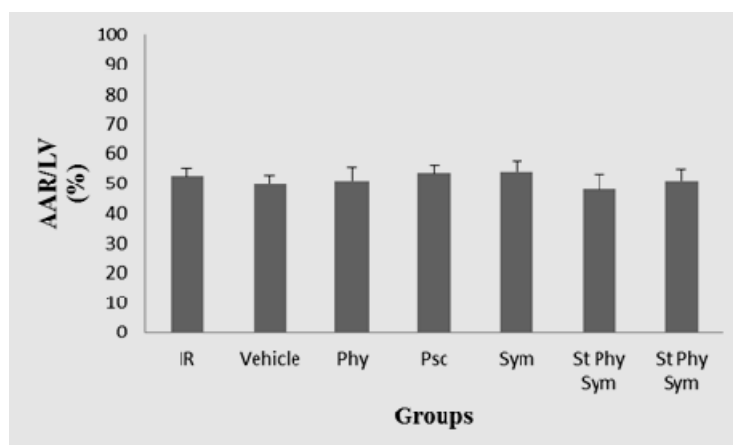


Figure 4. Area at risk in different groups (n=8)
 AAR: area at risk, LV: left ventricle, I/R: ischemia/reperfusion, SYM: control of sympathectomy, PHY: physical stress, PSC: psychological stress, PHY+SYM: Sympathectomy+physical stress, PSC+SYM: Sympathectomy+ psychological stress. Data are presented as mean ± S.E.M

The ratio of infarct size to area at risk (%IS) increased considerably from 32.8 ± 1.8 in IR group to 43 ± 2 in physical stress ($P < 0.05$) and 48 ± 2.4 in psychological stress group ($P < 0.01$). After sympathectomy, animals received physical and

psychological stress showed significantly reduced infarct size to 31.25 ± 1.4 and 34 ± 4.5 ($P < 0.05$), respectively vs. the physical and psychological stress groups, respectively (Figure 5).

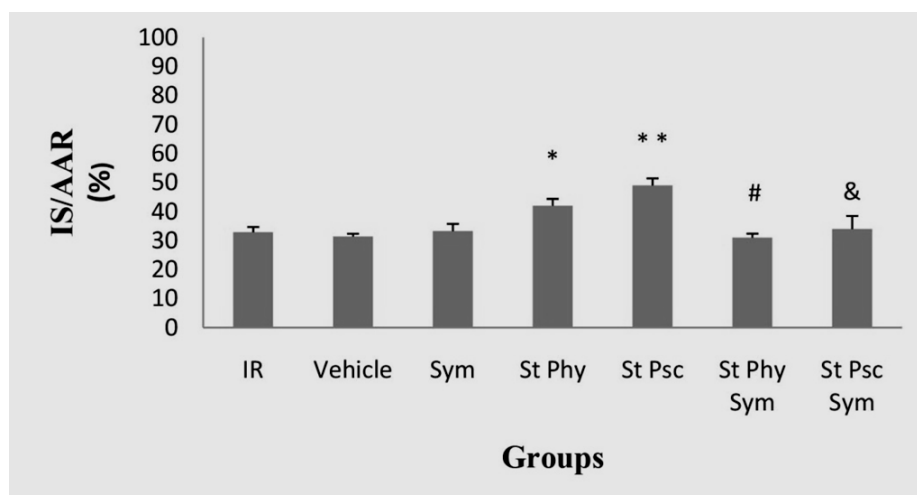


Figure 5. Myocardial infarct size in different groups (n=8)

IS: infarct size, AAR: area at risk, I/R: ischemia/reperfusion, SYM: control of sympathectomy, PHY: physical stress, PSC: psychological stress, PHY+SYM: Sympathectomy+physical stress, PSC+SYM: Sympathectomy+ psychological stress. Data are presented as mean \pm S.E.M.

* $P < 0.05$ compared to IR group. ** $P < 0.01$ compared to IR group. # $P < 0.05$ compared to physical stress, & $P < 0.05$ compared to psychological stress.

Corticosterone plasma levels

To establish the efficacy of communication box (stress box) in which used to stress induction, blood samples were obtained from control and stress groups and plasma levels of corticosterone was measured by

corticosterone kit (DRG, Marburg, Germany). Results showed that the amount of plasma corticosterone concentration in all stress groups (with or without sympathectomy) was significantly more than corticosterone concentration in IR group (Figure 6).

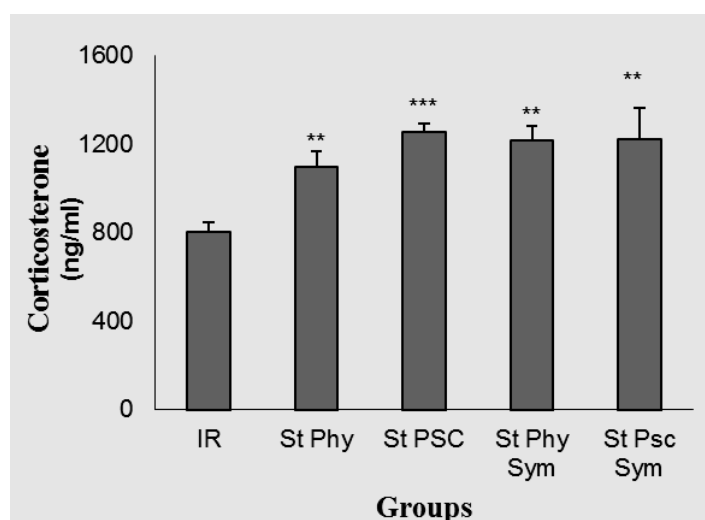


Figure 6. The amount of plasma corticosterone concentration in stress groups in comparison to I/R group.

I/R: ischemia/reperfusion, PHY: physical stress, PSC: psychological stress, PHY+SYM: Sympathectomy+physical stress, PSC+SYM: Sympathectomy+ psychological stress. ** $P < 0.01$ and *** $P < 0.001$ compared to I/R group

Systolic blood pressure

To evaluate the efficacy of sympathectomy drug (6-OHDA), animal's systolic blood pressure was measured by tail-cuff and PowerLab before and 24 hours after of

sympathectomy in non-invasive blood pressure (NIBP) method. Results showed that systolic blood pressure was significantly decreased by chemical sympathectomy ($P < 0.05$) (Figure 7).

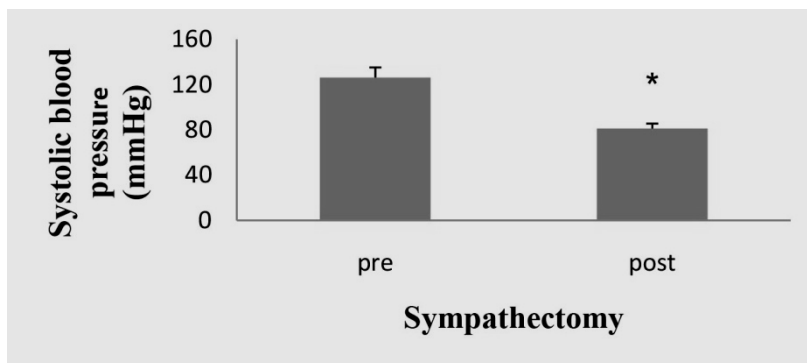


Figure 7. The comparison between systolic blood pressure before and after chemical sympathectomy with 6-OHDA. Data are presented as mean \pm S.E.M and student t-test was used for statistical analysis. * $P < 0.05$

Discussion

Current results indicated that the induction of chronic physical and psychological stress increased ischemia/reperfusion-induced injuries in isolated rat heart. There are paradoxical reports about the beneficial or harmful effects of stress on the body. In this regard, it is reported that chronic psychological stress increases blood pressure and attenuates the autonomic regulation of heart function (22). Also, psychological stress through activation of the sympathetic nervous system can lead to cardiovascular and metabolic disturbances (13). On the other hand, it has been shown that induction of cold restraint stress protects the rat heart against ischemia/reperfusion injury (23). In our previous study, induction of one episode of acute forced swimming stress prior to ischemia/reperfusion significantly decreased infarct size and preconditioned the isolated rat heart (24). Stress can generate many responses via activation of HPA axis and sympathetic nervous system and through blood pressure rise, insulin sensitivity drop (with or without hyperglycemia) and activation of intravascular coagulation can lead to cardiovascular dysfunction (25,26). In addition, a few minutes after stress, risk factors of myocardium ischemia such as sympathetic nervous system outflow, plasma level of catecholamines and corticosteroids, heart rate, blood pressure, coagulation factors and blood viscosity increases (27-29).

In this study, there are no significant differences in LVDP, RPP, and infarct size between chronic physical

and psychological stress groups and it seems that chronic physical and psychological stress have similar effects on the heart. These findings are supported by other studies in which physical and psychological stress had equal effects on acid and pepsin gastric secretion (30) and isolated aortic reactivity to vasoactive agents. In contrast, in another study, it has been reported that body temperature and motor activity in rats subjected to physical stress significantly increased in comparison with those which received psychological stress (31).

In addition, present results showed that the adverse effects of physical and psychological stress diminished by chemical sympathectomy and it is suggested that the activity of sympathetic nervous system is responsible for deleterious effect of chronic stress on heart. Previously it has been confirmed that hyperactivity of sympathetic nervous system is involved in some pathophysiological disease such as congestive heart failure (CHF). In CHF, chronic activation of the sympathetic system through an increase in peripheral vascular resistance and heart work attenuates the mechanical force of the heart (32). The long-term administration of catecholamines and β -agonist can accelerate all adverse events that occur in stress cardiomyopathy, but short-term stimulation of sympathetic nervous system via energy supplement and cardiac contractility enhancement increases the heart efficacy (33).

These results show that induction of chronic physical and psychological stress prior to ischemia/reperfusion causes enhancement of myocardial injuries and it seems that increased sympathetic activity in response to stress

is responsible for these adverse effects of stress on ischemic/reperfused heart.

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