ARTICLE ORIGINAL / ORIGINAL ARTICLE

CHRONIC SLEEP DEPRIVATION EFFECTS ON ISCHEMIA-REPERFUSION INJURY
Role of the Sympathetic Nervous System

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ABSTRACT • Objective: Chronic sleep deprivation has disturbing effects on health. The aim of this study is to assess the effects of chronic sleep deprivation on myocardial ischemia-reperfusion injury. **Methods**: Male Wistar rats were randomly divided into four groups; the heart of all the animals was perfused in the Langendorff apparatus using Krebs-Henseleit buffer. Hearts were allowed a 15-minute recovery which was considered as baseline, then underwent 30 minutes ischemia followed by 60 minutes reperfusion. Grouping: 1) Ischemia/reperfusion group (IR); 2) Control group (CON): rats were placed in large multiple platforms for 72 hours prior to IR; 3) Chronic sleep deprivation group (CSD): 72 hours sleep deprivation was induced using small multiple platforms prior to IR; 4) Sympathectomy group (SYMP): chemical sympathectomy was done 24 hours before chronic sleep deprivation induction followed by subjection to IR. Outcome measures included heart rate (HR), left ventricular developed pressure (LVDP), rate product pressure (RPP) and the ratio of infarct size to area at risk (IS/AAR %). **Results**: Our results showed IS/AAR considerably increased in the IR group as compared to the sleep deprivation group ($p < 0.001$); and sympathectomy could decrease infarct size as compared to sleep deprivation ($p < 0.001$). There were no significant differences between HR, LVDP and RPP between groups. **Conclusions:** Induction of 72 h sleep deprivation following ischemia and reperfusion increased the infarct size in a sympathetic dependent manner, and this effect of sleep deprivation is not related to changes in hemodynamic parameters.

Keywords : rats; chronic sleep deprivation; infarct size; myocardial ischemia reperfusion; sympathetic nervous system

INTRODUCTION

Adequate sleep is necessary for regulation of different biological systems. On the other hand increasing numbers of people are chronically sleep deprived (CSD) because of greater work pressure in urban economies and shift works [1].

Recently studies have shown that short sleep duration can lead to different pathological situations such as, imbalance in immune systems, thermoregulation and stroke [1-3]. Additionally chronic sleep restriction play an important role in the development of cardiovascular system diseases such as hypertension and sudden cardiac death [4,5]. Heart rate variability is an index of cardiac autonomic system activity. It describes the variations in the interval between consecutive heart beats and is caused by the continuous interplay between the efferent cardiac parasympathetic and sympathetic activities [6]. Some possible mechanisms include activation of sympathetic activity, endothelial dysfunction, decrease nitric oxide (NO) and its vasodilatation activity, redox imbalance and increasing of inflammatory cytokines [7]. Sleep deprivation adversely affects several systems that could change vascular response through sympathetic activation or inflammation [8] but the exact mechanism is not clear.

Therefore, the aim of this study was to assess the effect of chronic sleep deprivation induction before ischemia reperfusion (IR) on the myocardial injuries in isolated rat heart and the role of the sympathetic nervous system in this case.

MATERIAL AND METHODS

Experimental animals and ethical approval

Male Wistar rats (n = 4-10) weighing 250-300 g were kept in the animal house with 12 h light/dark cycles at 22 ± 2°C and free access to food and water. The experimental protocols followed in this study were conformed to the Guidelines for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication No. 85-23, revised 1996) and were further approved by the institutional ethical committee of Tehran University of Medical Sciences (Tehran, Iran).

Preparation of isolated hearts

The animals were anesthetized with sodium pentobarbital (60 mg/kg i.p.) and given heparin sodium (500 IU/kg) to prevent blood coagulation. When the animal was deep anesthetized, the heart was exposed and excised quickly, then attached to Langendorff apparatus for perfusion. Hearts were perfused with Krebs-Henseleit bicarbonate buffer containing: $NaHCO_3$ 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 and 5% CO_2 (pH = 7.35-7.45 at 37°C). A latex, fluid-filled, isovolumic balloon was introduced into the left ventricle through the left atrial appendage and distended to give preload of 8 to 10 mmHg and connected to a pressure transducer (Harvard Apparatus, Holliston, MA, USA). A surgical needle was passed under

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the origin of the left anterior descending coronary artery, and the ends of the structure were passed through a pipette tip to form a snare. Regional ischemia was induced by tightening the snare and reperfusion was performed by releasing the ends of the structure. The perfusion apparatus was water-jacketed to maintain a constant perfusion temperature of 37ºC.

Hearts were allowed to beat spontaneously throughout the experiments.

Experimental protocol

All animals were randomly divided into four groups:

1. Ischemia/Reperfusion group (IR): 30 minutes ischemia followed by 60 minutes induced reperfusion (n = 7);
2. Control group (CON): rats were placed in large multiple platforms for 72 h prior to ischemia and reperfusion (n = 6);
3. Chronic sleep deprivation group (CSD): 72 h sleep deprivation was induced by using small multiple platforms prior to ischemia and reperfusion (n = 10);
4. Sympathectomy group (SYM): chemical sympathectomy was done 24 h before chronic sleep deprivation and followed by ischemia and reperfusion (n = 4).

Hemodynamic functions

Hemodynamic parameters including heart rate (HR), beats per minute (bpm), and left ventricular developed pressure (LVDP) in millimeters of mercury (mmHg) were monitored with a software (Ossilo Graph Monitor, Biomed, Tehran, Iran). The pressure transducer was connected to a computerized chart recorder system to record various hemodynamic parameters including heart rate, left ventricle developed pressure as an index of systolic function and rate pressure product (RPP) = heart rate x left ventricular developed pressure.

Modified multiple platform method

Sleep deprivation was induced in a water tank (125 x 44 x 44 cm) containing 8 round platforms of 6/5-cm-diameter (small platform) or 14-cm-diameter platforms (large platform). Large platforms are often used as a control group in sleep deprivation experiments where rats could sleep without falling into the water.

The tanks were filled with water approximately 1 cm below the surface of the platforms which were arranged in two rows and rats could move from one platform to another. Upon sleeping, the animals would fall into the water due to muscular atonia and would awake. Rats had free access to water and food hanging from the aquarium cover in a climate controlled room (22-24°C) on a 12/12 h light/dark cycle [8].

Infarct size measurement

After completion of the reperfusion period, the left coronary artery (LAD) was reoccluded, and Evans blue dye (0.3 - 0.5 mL) was infused through the aorta to differentiate the ischemic zone from non-ischemic zone. Hearts were frozen and then cut into 2 mm slices, using stainless steel rat heart slicer matrix with 2 mm coronal section slice intervals, transverse sections from apex to base. Slices were then incubated with 1% triphenyltetrazolium chloride (TTC) in 0.1 M phosphate buffer, pH = 7.4, for 20 min at 37ºC. TTC reacts with viable tissue, producing a red formation derivative which is distinct from the white necrotic tissue once fixed in 10% formalin for 24 h. The areas of the left ventricle, area at risk (AAR), and infracted tissue were measured by method of planimetry from the scanned hearts by using Photoshop program. AAR was expressed as a percentage of left ventricle size for each heart and the infarct size (IS) was expressed as a percentage of AAR [9,10].

Chemical sympathectomy

Chemical sympathectomy was performed by injection of 6-hydroxydopamine (100 mg/kg; Sigma-Aldrich, USA) diluted in NaCl 9% and ascorbic acid 1% (Daroupakhsh Co. Iran), subcutaneously [11].

Measurement of plasma epinephrine

After anesthesia, before heart isolation, blood samples were collected. The samples were centrifuged (2000 rpm, 15 min at 4°C) and the plasma was removed and frozen until assessed.

Plasma epinephrine level was measured using a specific ELISA kit (R&D, Minneapolis, MN, USA) according to the manufacture instructions.

Statistical analysis

Statistical analysis of all outcome measurements within and between groups was performed with ANOVA (one-way analysis of variance) followed by Tukey's post-hoc test. Changes in plasma level of epinephrine comparison between groups were done by unpaired t-test. All the analyses were performed using the SPSS software, version 20 (SPSS Inc., Chicago, IL, USA).

All values were expressed as the mean ± standard error of mean (SEM). Statistical significance was defined as $p < 0.05$. Prespecified sample size from previous studies was used to obtain significant results.

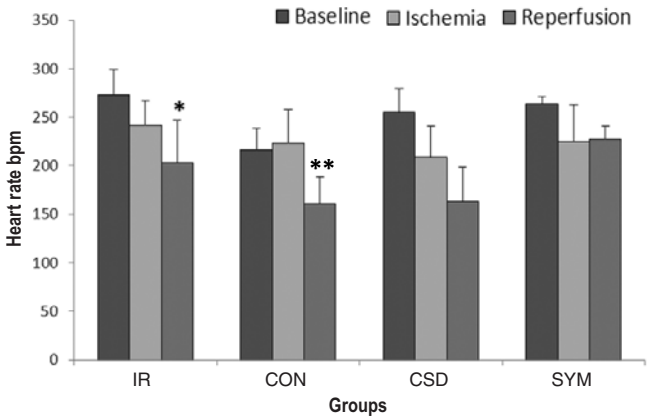
RESULTS

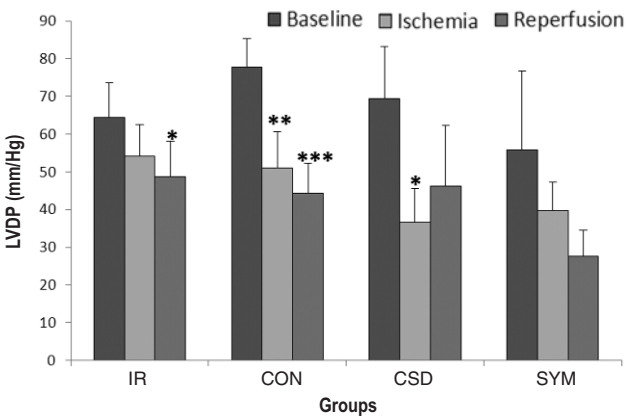
Hemodynamic data

Heart rate (HR), left ventricular developed pressure (LVDP) and rate pressure product (RPP) changes during the different periods of experiment are shown in Figures 1, 2 and 3.

The results depicted in diagram show HR, LVDP and RPP decreased in all groups at the end of ischemia and reperfusion compared to their baseline.

There were no significant differences of hemodynamic parameters in baseline, ischemia and reperfusion periods among groups (one-way ANOVA and repeated measure tests).





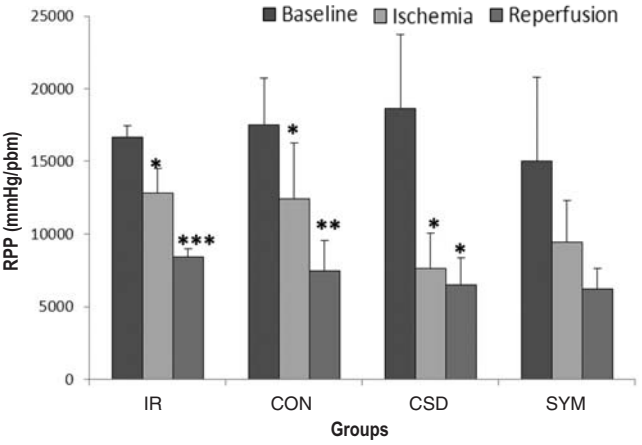
bpm: beat per minute **LVDP**: left ventricle developed pressure **IR**: ischemia reperfusion group
CON: control group **CSD**: chronic sleep deprivation group **SYM**: sympathectomy group

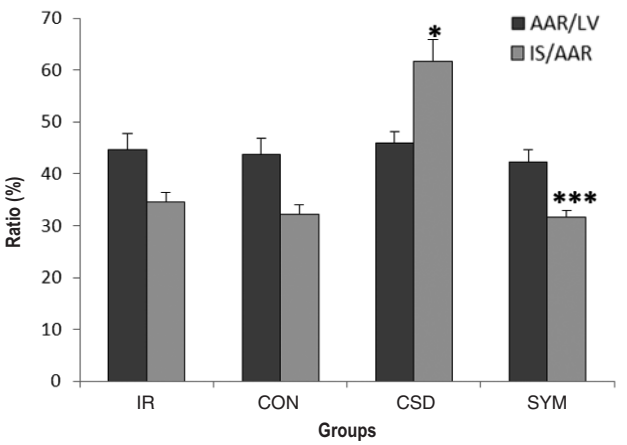
Figure 1

Heart rate at baseline, ischemia & reperfusion periods
***p** < 0.05 when compared with baseline
****p** < 0.05 when compared with ischemia

Figure 2

Left ventricle developed pressure at baseline, ischemia & reperfusion periods
When compared with baseline: ***p** < 0.05 ****p** < 0.01 *****p** < 0.001





RPP: rate product pressure **IR**: ischemia reperfusion group **CON**: control group **CSD**: chronic sleep deprivation group
SYM: sympathectomy group **AAR/LV %:** area at risk/letf ventricle % **IS/AAR %:** infarct size/area at risk %

Figure 3

Rate product pressure at baseline, ischemia & reperfusion periods
When compared with baseline: ***p** < 0.05 ****p** < 0.01 *****p** < 0.001

Figure 4

Myocardial area at risk expressed as a % of the letf ventricle & myocardial infarct size expressed as a % of the area at risk
When compared with IR group : ***p** < 0.05 *****p** < 0.001

Area at risk and infarct measurement

As shown in Figure 4, there were no statistical differences in the ratio of the area at risk to total left ventricular area between the hearts in all experimental groups. The ratio of infarct size to area at risk in CSD group (61.7 ± 4.2) was significantly increased as compared to IR group (34.5 ± 1.98) and this ratio was significantly decreased in SYM group (31.7 ± 1.2) as compared to CSD group ($p < 0.001$).

Plasma level of epinephrine

The result of the assessment of epinephrine concentration in the serum, shown in Figure 5, revealed that there was no difference between IR and CSD groups. Epinephrine level was significantly lower in SYM group as compared to CSD group ($p < 0.05$). 0

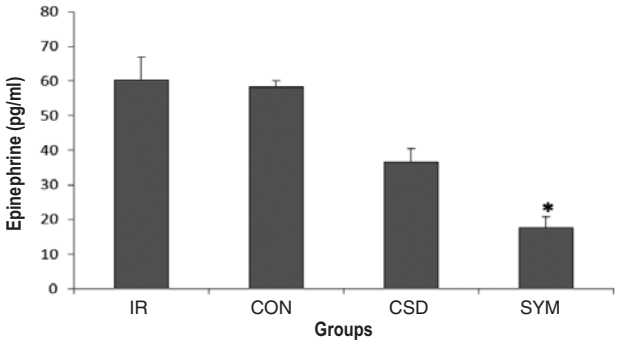


Figure 5

Epineprine concentration in serum
***p** < 0.05. when compared with IR group

DISCUSSION

There are extended studies to suggest the relation between sleep deprivation and arrhythmia but to the best of our knowledge, it is the first study to investigate the effect of chronic sleep deprivation on the infarction size.

Our results demonstrated that there was a significant relation between chronic sleep deprivation and increasing of infarct size, but no significant differences were seen between sleep deprivation and hemodynamic variables (HR, LVDP and RPP). The first hypothesis for explaining this relation is that sleep deprivation can lead to increasing sympathetic activity and consequently cardiac workload, therefore an elevation in O_2 demand, which results in enlarging the infarct size.

In this regard, Dettoni *et al*. revealed that sleep deprivation can increase sympathetic activity and endothelial dysfunction [12]. Increasing autonomic nervous system (ANS) activity in different pathways can contribute to enhancing myocardial ischemia-reperfusion injuries, such as increasing vascular resistance, blood pressure (BP) and endothelial dysfunction and also lead to decrease nitric oxid (NO) bio-availability that is one of the most potent vasodilator factors [12,13].

Increasing sympathetic activity during the wakefulness period, due to decrease in total sleep time can lead to transient rise in blood pressure and heart rate. Sympathetic activity enhancement has been involved in the decrease of endothelial-dependent vasodilation and suspected to link sleep deprivation and endothelial dysfunction. However, these results show for the first time that endothelial dysfunction induced by total sleep deprivation persists in sympathectomized rats [8].

Considering the role of sympathetic nervous system in cardiovascular function, in this study we assessed the effect of chronic sleep deprivation on hemodynamic parameters affected by heart ischemia reperfusion and infarct size and investigate the association between chronic sleep deprivation, sympathetic and infarction size.

Sympathectomy has been done and results showed that infarct size decreases in sympathectomy group, but the results of hemodynamic parameters in SYM group were not significantly lower (RPP and LVDP) and higher (HR) than in other groups. Since it has been confirmed that vessels are sympathetically innervated and the heart is more affected by sympathetic nervous system, sympathectomy would remove the proper answer of cardiovascular system to situations which effect on hemodynamic parameters such as LVDP, HR and RPP. According to some investigators, vasodilation after sympathectomy, led to the decline of heart rate and cardiac output which reflected decreasing of tone and muscle movement [14]. Furthermore, Nonomura *et al*. have demonstrated that cardiac sympathectomy can improve ischemic situation by reducing O_2 demand [15]. Rising in ANS activity has been shown in some other researches by measuring the epinephrine plasma level. Takase *et al*. assessed plasma levels of epinephrine in students after four weeks examination periods and observed that its significant rise could be due to experiencing both sleep deprivation and stress [13].

Moreover, blood pressure increases as a result of sympathetic overactivity play an important role in these events. Chouchou *et al*. revealed that sleep fragmentation by overactivation of sympathetic system can contribute to systolic hypertension and other major confounders such as sleep disorder breathing (SDB), hypoxemia, diabetes and hypercholesterolemia are all involved in major cardiovascular risk factors [16].

These data suggest that processes such as reduced sleep duration and circadian shift, in addition to elevated neuroendocrine stress in shift workers could affect the 24 h-blood pressure profile and potentially predispose to hypertension [17]. According to possible mechanisms linking sleep disorders to cardiovascular diseases, many studies have observed that chronic sleep disorders or acute sleep restriction are associated with activation of the sympathetic nervous system, increased blood pressure, and baroreflex sensitivity changes in both humans and rodents [8].

Recently, studies have shown that sleep deprivation with increased levels of inflammatory cytokines such as C-reactive protein (CRP), tumor necrosis factor alpha (TNFα) and interleukin 4 (IL-4) leads to endothelial dysfunction and impairs NO availability by Cyclooxygenase-2 (COX-2) dependent pathway that results in increasing the production of oxidative stress and infarction [8].

To explain how sleep deprivation can decrease NO bioavailability, Sauvet *et al*. suggest that a five-day sleep restriction decreases nitric oxide synthase (NOS) availability in different parts of brain and cardiovascular efferent neurons that cause to increase inhibitory effects on regulation of cardiovascular system [8]. The high mortality of sympathectomized animals in sleep deprivation induction justifies the small sample size for this group

CONCLUSION

It seems that chronic sleep deprivation effects on infarct size in a sympathetic dependent manner do not bring about any change in hemodynamic parameters.

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