Effect of Acute Sleep Deprivation on Ischemia-induced Ventricular Arrhythmia in the Isolated Rat Heart

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Abstract
Background and Objective: Sleep deprivation (SD) is caused by a host of reasons and has numerous consequences on cardiac system. In this study, we aimed to assess the preconditioning effects of acute SD on ischemia (IS)-induced ventricular arrhythmias in isolated rat hearts.

Materials and Methods: Male Wistar rats randomly were placed into the four groups: IS-group, acute SD group, control group for SD, and sympathectomy group (SYM). SD paradigm in SD was performed 24 hours before IS induction. In SYM groups, the animals were chemically sympathectomized 24 hours before SD. The rat hearts were isolated and perfused with Krebs buffer solution by Langendorff method and subjected to 30 minutes regional IS. Throughout the experiment, the hearts were allowed to beat spontaneously; thereafter, heart rate (HR) and ventricular arrhythmias were measured.

Results: No differences were found between the experimental groups for HR at baseline and IS period. As compared to IS group, SD animals showed less incidence of ventricular tachycardia and severity of arrhythmias (P < 0.050). Furthermore, significantly the number of ventricular ectopic beats episodes/min, bigeminy/min, trigeminy/min, and couple/min were less than IS group (P < 0.050). Moreover, sympathectomy could reverse results to IS group level as compared to sleep deprived animals (P < 0.050).

Conclusion: It is concluded that induction of acute SD before IS could reduce ventricular arrhythmias, and chemical sympathectomy removed this cardioprotection.

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Keywords: Sleep Deprivation; Ischemia/Reperfusion; Ventricular Arrhythmia; Isolated Rat Heart

Introduction
Sleep through circadian rhythms has a significant role for regulating of the cardiac sympathetic nervous system activity than the parasympathetic nervous system. Increased cortisol levels, decreased glucose tolerance, learning and memory impairments, increased sympathetic nervous system activity, activation of the hypothalamic–pituitary–adrenal axis, and increased blood pressure are the main consequences of sleep deprivation (SD) (1-4). In addition to the influences of SD on basic activities, it affects the responses to stress (4).

Ischemic heart disease is the most common cause of mortality and for this reason, the treatment protocol can be important and valuable for reducing myocardial ischemic injuries (3, 5). Myocardial Infarction (MI) occurs when myocardial ischemia (IS) exceeds a critical
threshold and abolishes the myocardial cellular repair mechanisms for maintaining normal cardiac function and homeostasis (6). The heart can be protected by application of many chemical or physical stimuli, such as adrenoreceptors activation with norepinephrine, before long-term IS. This phenomenon is called preconditioning that causes reduction of cardiac injuries and mortality (7). Although reperfusion of ischemic cardia is effective in reduction of infarction size, it can cause cardiac damages. Intensity and duration of sympathetic nervous system activation can affect the extent of injuries in ischemic heart. Long-term activation of the sympathetic nervous system increases ischemic injuries and short-term activation of the heart by norepinephrine and phenylephrine before IS reduces these damages (8).

To our knowledge, there is no study addressing the beneficial consequences of acute SD on ischemic heart and this study evaluates the preconditioning effect of acute SD and the role of the sympathetic nervous system in this regard on IS-induced ventricular arrhythmias.

Materials and Methods

Animals

Male Wistar rats (200-250 g) were kept in an air-conditioned colony room on a light/dark cycle at 21-23 °C with free access to food and water. 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.

Preparation of isolated hearts and induction of regional IS

The animals were anesthetized with sodium pentobarbital (60 mg/kg, i.p., Sigma, Steinheim, Germany). The hearts were excised and immediately aorta was cannulated for retrograde perfusion by Langendorff apparatus with Krebs buffer containing (in mmol/l): NaHCO\(_3\) - 25; KCl - 4.7; NaCl - 118.5; MgSO\(_4\) - 1.2; KH\(_2\)PO\(_4\) - 1.2; glucose - 11; CaCl\(_2\) - 2.5 gassed with 95% O\(_2\) 5% CO\(_2\) (pH: 7.35-7.45 at 37 °C). Afterward two thin stainless steel electrodes were fixed on the ventricular apex and right atrium for recording electrocardiogram (ECG) lead II. At the end of surgical procedure and recovery period, 15 minutes of baseline was considered. A surgical needle (5-0 silk suture) was passed around the origin of the left anterior descending coronary artery (LAD), and the ends of the suture were passed through a pipette tip to form a snare. By tightening the snare, regional IS was induced. IS was showed by ST elevation and increase in amplitude of R-wave in ECG. To maintain constant perfusion temperatures of 37 °C, the perfusion apparatus was water-jacketed. Hearts were allowed to beat spontaneously throughout the experiments. The heart rate (HR; beats/min) as one of the hemodynamic parameters was calculated through ECG. During the recovery period, any rat with ventricular fibrillation (VF) lasting for more than 5 minutes was discarded from the study.

SD paradigm

About 24 hours’ SD was induced using a metal tank (125 cm × 44 cm × 44 cm) containing 8 circular platforms (6.5 cm in diameter). The tank was filled with water (with a temperature of 20 °C) until approximately 1 cm from the platforms surface. The rats were allowed to move around freely inside the tank by jumping
from one platform to another. When they entered the paradoxical phase of sleep, the rats were awakened due to falling into the water as a result of muscle atonia. Food and water were provided ad libitum by placing chow pellets and water bottles at the top of the tank (5, 6). Another tank containing four platforms 14 cm in diameter was used to establish an environmental control group for SD (SD-CON). The rats could sleep on these platforms without falling into the water.

**Chemical sympathectomy (SYM)**

Chemical sympathectomy was performed by subcutaneous injection of 6-hydroxydopamine (100 mg/kg) 24 hours before acute SD (9).

**Study design and experiment group**

The animals were divided into four groups and all of them underwent 30 minutes of regional IS:

I.IS-group (n = 7): hearts underwent regional IS

II.Acute SD group (n = 11): 24 hours SD was induced and then the hearts underwent regional IS

III.SD-CON (n = 9): animals were placed in a water tank containing large platforms for 24 hours prior induction of regional IS

IV.SYM group (n = 6): 24 hours after chemical sympathectomy, the rats endured 24 hours of sleep deprived and then the hearts underwent IS.

**Assessment of ventricular arrhythmias**

IS-induced ventricular arrhythmias were defined based on Lambeth conventions (10). Ventricular ectopic beats (VEBs) were determined as identifiable premature QRS complexes. Other multipart forms of VEBs such as bigeminy (periodical consecutive normal QRS and VEB), couplet (two consecutive VEBs), and triplet (three consecutive VEBs) have been considered for calculating VEB/min. Ventricular tachycardia (VT) was determined as the appearance of four or more consecutive VEBs at a rate faster than the resting sinus rate. Unidentifiable and low voltage QRS complexes were defined as VF.

Severity of arrhythmias was scored according to the following criteria:

- 0- Hearts with 0-50 VEBs;
- 1- 50-500 VEBs;
- 2- More than 500 VEBs; or one episode of spontaneously reversible VT or VF
- 3- 2-30 episodes of spontaneously reversible VT and/or VF;
- 4- More than 30 episodes of spontaneously reversible VT and/or VF;
- 5 - Irreversible VF (11).

**Statistical analysis**

The data are expressed as a mean ± standard error of mean. The statistical analysis of ventricular arrhythmias during IS was performed by one-way ANOVA between all groups and a subsequent Tukey’s test as needed. Comparison of HR during baseline and IS periods between groups was done by two-way ANOVA test. The arrhythmia scores were analyzed with Kruskal–Wallis test, and the incidence of VT was compared using the Fisher exact test. Analyses were performed using the SPSS software, (version 20; SPSS Inc., Chicago, IL, USA) and any P < 0.050 was considered as statistically significant.

**Results**

HR as a cardiac function parameter is presented in table 1. No differences were found between the experimental groups for HR at baseline period. Moreover, there
was no difference between baseline and IS periods within each group.

Table 1. Values of cardiac functional parameters (HR)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Baseline</th>
<th>IS</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IS</td>
<td>172.66 ± 26.28</td>
<td>277.28 ± 18.45</td>
<td>0.256</td>
</tr>
<tr>
<td>SD</td>
<td>221.38 ± 24.22</td>
<td>293.61 ± 13.49</td>
<td>0.651</td>
</tr>
<tr>
<td>SD-CON</td>
<td>193.66 ± 32.59</td>
<td>233.44 ± 24.33</td>
<td>0.730</td>
</tr>
<tr>
<td>SYM</td>
<td>210.00 ± 27.49</td>
<td>262.16 ± 23.56</td>
<td>0.082</td>
</tr>
</tbody>
</table>

IS: Ischemia, SD: Acute sleep deprivation group, SD-CON: Control group for sleep deprivation, SYM: Sympathectomy group. P < 0.050: Significant difference between baseline and ischemia, HR: Heart rate

In this study, regional IS with occlusion of LAD coronary artery produced rigorous ventricular arrhythmia. In SD group, both VEB/min (14.94 ± 0.74 vs. 24.73 ± 1.02; P < 0.001) and score of arrhythmias (1.25 ± 0.16 vs. 2.42 ± 0.20; P = 0.001) were less than IS group; sympathectomy in SYM group (28.52 ± 1.20; P = 0.069 and 2.67 ± 0.21; P = 0.829, respectively) returned them to IS group level (Figure 1).

![Figure 1](image1.jpg)

**Figure 1.** Score of arrhythmias and number of episodes of ventricular ectopic beats (VEB/min) during 30 minutes ischemia (IS). IS, Acute sleep deprivation group (SD), control group for sleep deprivation (SD-CON) and sympathectomy group (SYM); *P < 0.050 versus IS group and ¶P < 0.050 versus SD group

According to figure 2, sleep deprivation in SD-group could reduce bigeminy (1.60 ± 0.25), trigeminy (0.72 ± 0.04), and couple per minute (0.64 ± 0.06) compared to IS group (2.87 ± 0.21; P = 0.061, 26.00 ± 0.08; P = 0.006; and 1.13 ± 0.07; P = 0.014, respectively); sympathectomy eliminated these cardioprotective effects in SYM group, too (3.89 ± 0.37, 2.21 ± 0.16, and 1.97 ± 0.22, respectively).

![Figure 2](image2.jpg)

**Figure 2.** Number of bigeminy per minute (bigeminy/min), trigeminy per minute (trigeminy/min), and couple per minute (couple/min) during 30 minutes ischemia (IS). IS, acute sleep deprivation group (SD), control group for sleep deprivation (SD-CON), and sympathectomy group (SYM); *P < 0.050 versus IS group and ¶P < 0.050 versus SD group

As illustrated in figure 3, compared to IS group, application of 24 hours SD before IS reduced significantly the incidence of VT (9.09% for SD vs. 42.85% for IS group; P = 0.018). This protection of SD was removed by chemical sympathectomy in SYM subjects (66.67%; P = 0.001), also incidence of VT in SYM group was more than IS group (P = 0.033). In addition, comparison of VT incidence between IS and SD-CON groups (44.45%; P = 0.582) did not show any significant difference.

**Discussion**

Sleep is a physiological process, through which the activation of several cortical and subcortical neural networks occurs. The complex and bidirectional relation between sleep and cardiovascular system are that sleep disorders may affect cardiovascular system and increase risk of
cardiovascular problems, while, on the contrary, physiological sleep alters in cardiovascular diseases (12).

Based on controlled chronobiologic studies, it is shown that sleep was more important for sympathetic nervous system regulation of the heart in comparison with the parasympathetic system (13). Inadequate sleep and sleep disturbance may co-occur with sympathetic hyperactivity (10); therefore, we hypothesized that sleep disturbance can be related to the adrenergic overdrive.

In the current study, we aimed to evaluate the effect of acute SD before MI on ventricular arrhythmia in isolated rat hearts. Moreover, in this study to determine the association between SD and cardiovascular dysfunction, some of the experimental animals were sympathectomized by injection of 6-hydroxydopamine.

We observed that application of 24 hours SD prior IS, preconditioned the myocardium against ventricular arrhythmias, and it seems that this protection has occurred through sympathetic nervous system activation because sympathectomy before SD inhibited these preconditioning responses.

The autonomic nervous system affects modulation of cardiac electrophysiology and arrhythmogenesis. Tejero-Talido et al. (14) found that α1-adrenoceptor stimulation produces late preconditioning in mouse heart. We previously have shown that administration of noradrenaline decreases IS/reperfusion insult (11) and blocking of mitochondrial KATP channels before or after administration of noradrenaline leads to inhibition of myocardium preconditioning (3). Moreover, it is shown that a kind of selective blocker of presynaptic α2 adrenoceptor known as yohimbine can increase release of endogenous noradrenaline which leads to reduction of arrhythmia severity (15). It was also reported that β-blockers induce cardioprotection via permitting norepinephrine to activate α1 adrenoceptors, accompanied by decline in mortality and morbidity due to MI (16).

It is mentioned previously that not only pump failure, but also cardiac arrhythmias are connected with changes of HR (11, 17). In the present study, there was no difference in the HR during IS period among the groups and it seems the incidence of arrhythmias in our experimental groups is not related to the HR changes. Although there were no differences in HRs among the experimental groups, the results showed that SD exhibited cardioprotective effects during IS, evidenced by reduced ventricular arrhythmias in SD animals as compared to IS group. The causal relationship between changes in sympathetic nerve activity and the development of cardiac arrhythmias has been demonstrated in which acute SD by sympathetic activation probably provides
protection for the heart and reduces ventricular arrhythmia.

This study is the first to show that acute SD can induce early preconditioning against IS-induced ventricle arrhythmia via activation of sympathetic nervous system; however, the lack of hemodynamic reflexes and neurohormonal effects in isolated heart using a longendorff system seems to be a limitation of this study to assess more details in this regard.

Conclusion

This study adds the notion that application of 24 hours’ SD prior IS reduces incidence and severity of arrhythmias, and chemical sympathectomy abolished the beneficial effects of acute SD on isolated ischemic rat heart. Finally, further research is needed to determine the main preconditioning mechanisms of acute SD before MI which can affect the incidence of arrhythmias after infarction.

Conflict of Interests

Authors have no conflict of interests.

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References


