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Original Article

Susceptibility to life-threatening ventricular arrhythmias in an animal model of paradoxical sleep deprivation

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ABSTRACT

Background: According to some reports regarding the increase of cardiac events following sleep deprivation, our study was conducted to clarify the effects of rapid eye movement (REM) sleep deprivation on susceptibility to lethal ventricular arrhythmias in rat.
Methods: The animal groups included the control group; the sham 48 and sham 72 groups (without sleep deprivation); and the test 48 and test 72 groups, who experienced REM sleep deprivation for 48 h and 72 h, respectively. For induction of cardiac arrhythmia, aconitine was infused via the tail vein of the animals.
Results: After 72 h of REM sleep deprivation, the blood pressure (BP) levels and the QTc interval of the electrocardiogram (ECG) were significantly increased ($P < .05$ and $P < .01$, respectively). However, the sleep deprivation had no significant effect on the heart rate (HR), myocardial oxygen consumption index, and plasma corticosterone level. Furthermore, sleep deprivation increased the latency times of premature ventricular contraction (PVC), ventricular tachycardia (VT), and also the PVC number; however, it did not increase the number, duration, and severity of VT and ventricular fibrillation (VF).
Conclusion: Our findings suggest that 72 h of REM sleep deprivation is associated with increased risk for hypertension and QT interval prolongation under nonstressful conditions; however, it does not increase the susceptibility to lethal ventricular arrhythmia in rat.

1. Introduction

Nowadays, individuals live in so-called sleep-deprived societies. Evidence shows that sleep time has been reduced to1.5 h per day and this decline is a continuing trend [1]. Increase in environmental light, long work days, shifts and night work, as well as the emergence of television, radio, and Internet use in individuals' lives are some responsible factors affecting the sleep duration [2]. In addition, some sleep disorders such as obstructive sleep apnea (OSA) are associated with partial sleep deprivation [3]. Moreover, OSA and most sleep deprivation occur during the paradoxical stage of sleep [4,5]. Epidemiologic studies reported a U-shaped relationship between sleep duration and mortality by which it was concluded that both sleep excess and sleep deprivation are a threat to survival [6]. Short sleep duration is associated with an increased risk for cardiovascular diseases and diabetes mellitus [6,7]. Although the exact mechanism is not clear, redox imbalance [8], elevation of endothelin levels [9], alteration in activity of the sympathetic nervous system [10,11], impairment of endothelium-dependent vasodilation [12], and activation of inflammatory processes [13–15] are some possible reasons. There are some speculations about the increased risk for cardiac arrhythmias in sleep-deprived individuals in the literature, and it has been reported that sleep deprivation may contribute to the development or recurrence of arrhythmias [16,17]. Still this association property is not clear, and to the best of our knowledge no study in the literature has directly investigated the effects of sleep deprivation on the development of lethal cardiac arrhythmia. Therefore, our study aimed to challenge the validity of the proarrhythmic effect of sleep deprivation and clarifying the effects of rapid eye movement (REM) sleep deprivation on blood pressure (BP) and also the susceptibility to lethal ventricular arrhythmias using an animal experimental model.

2. Material and methods

Our study was conducted according to the national guidelines for animal studies (Ethical Committee of the Kerman Neuroscience

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Research Center (EC/KNRC/90/6 – Kerman University of Medical Sciences). We included 80 male Wistar rats aged 3 months that weighed 250–350 g and were housed in a temperature-controlled room and were allowed free access to rat regular diet and water. Animals were randomly divided into five groups with 16 animals in each group as follows: (1) the control group (CTL), which were maintained in home cages without sleep deprivation; (2) two sham groups (i.e., S48 and S72), which were kept in a wide platform for 48 h and 72 h, respectively, without sleep deprivation; and (3) two paradoxical sleep-deprived groups (i.e., T48 and T72), which were kept in small platform for 48 h and 72 h, respectively.

2.1. Paradoxical sleep deprivation (REM sleep deprivation)

Paradoxical sleep deprivation (PSD) was applied by a multiple platform model. The apparatus contained a water chamber (90 cm × 50 cm × 50 cm) and two parallel rows (10 cm apart, edge to edge) of 10 circular platforms (10 cm [height], 7 cm [diameter], rising 2 cm above the water surface). When the animals (four rats) were placed within the multiplatform chamber, they moved freely with the least immobilization stress [18]. At the onset of each REM episode the animals were touched or fell into the water due to the loss of muscle tone, and thus were awakened. To test the possible environmental stresses, we also used a wide platform (15 cm [diameter]), which allowed the rats to sleep without falling into the water. The environment of apparatus during the experiments was controlled similar to cages with temperature (23 ± 1 °C) and a light–dark cycle (lights on, 07:00 am–7:00 pm), with free water access and chow pellets hanging from the top of the chamber [19].

2.2. Measurement of plasma corticosterone

At the end of the experiment (between 8:10 am and 8:30 am) under deep anesthesia, six animals of each group were killed and blood samples were collected. Then samples were centrifuged at 4 °C for 15 min at 2600*g* for isolation of plasma. The level of corticosterone in each sample was assessed by an ELISA kit specific to rat and mice (DRG International Inc., USA) with 0.25 ng/mL sensitivity [20].

2.3. Measured and calculated parameters

Ten rats from each group were considered for the assessment of hemodynamic parameters and arrhythmia susceptibility. The animals were anesthetized with intraperitoneal sodium thiopental (50 mg/kg) [21]. The right common carotid artery was cannulated by a filled polyethylene 50 tube (saline with heparin 15 IU/mL), which was connected to a pressure transducer and a PowerLab system (AD Instruments, Australia); the heart rate (HR) and arterial BP were continuously recorded during the experiment. The trachea was cannulated and animals were artificially ventilated with room air at 50 strokes per minute (stroke volume, 0.8 mL/100 g of body weight) during arrhythmia induction. The electrodes of electrocardiogram (ECG) lead two were attached to the limbs of the animals. An angiocath with gauge 24 was inserted into the lateral vein of the animal tail and then connected to a syringe containing arrhythmogenic drug (aconitine) by an appropriate tube. The time window for the animal recovery from surgery was 15 min, and the basal ECG and BP were recorded following recovery. The animals with cardiac arrhythmia or with a sustained drop in mean arterial BP below 70 mmHg during the stabilization period were excluded from the study.

The mean arterial pressure (MAP) was calculated using the MAP = Pd + (Ps − Pd)/3 formula, in which Pd is the diastolic arterial pressure and Ps is the systolic arterial pressure. Pressure-rate product (PRP), an indirect measure of myocardial oxygen demand, was determined as the product of the HR and mean arterial pressure ($[MAP^{*}HR]^{*}1000^{-1}$). The PR and QT interval of basal ECG in each group was determined by a mean of 1 min of ECG-recorded strip. Corrected QT (QTc) interval was measured using Bazett's formula normalized as $QTcn\text{-}B = QT/(RR/f)^{1/2}$, in which RR is R–R interval and f = 150 ms [22,23].

2.4. Arrhythmia induction

After basal recording of hemodynamic and ECG for arrhythmia induction, aconitine (from Sigma, England) was infused in the tail vein with a microinfusion pump at a velocity of 0.1 mL per minute (15 μg/mL in saline) [24] for ten minutes. The BP and ECG were simultaneously recorded during the infusion, and this process continued for another 5 min after the infusion period was over. During the 15 min of the experiment, the episodes of premature ventricular contraction (PVC), salvo, and ventricular tachycardia (VT) and ventricular fibrillation (VF) were counted and the latency and duration of PVC, VT, and VF were measured in seconds.

According to the Lambeth conventions, ventricular arrhythmias were defined as premature ventricular beats (PVB) or PVCs, discrete and identifiable premature QRS complexes, salvo, two or three consecutive PVBs, VT, a run of four or more consecutive PVBs, or VF, which were all signals of when individual QRS deflections could not easily be distinguished from each other and when the rate could no longer be measured [25]. The threshold dose of aconitine required for producing different ventricular arrhythmias (e.g., PVC, VT, VF) was determined according to the following formula: threshold (μg/kg) for arrhythmia = 15 μg/mL × 0.1 mL/minutes × time required for arrhythmia (min)/body weight (kg) = 1.5 μg/minutes × time (min)/body weight (kg). In addition, the severity of arrhythmias in the different groups was quantitatively presented by a scoring system [23] (0, <10 PVCs; 1, ⩾10 PVCs; 2, 1–5 episodes of VT; 3, >5 episodes of VT or 1 episode of VF; 4, 2–5 episodes of VF; and 5, >5 episodes of VF).

2.5. Statistical analysis

The results were presented as mean ± standard error of the mean. Comparison of corticosterone levels, HR, BP, and PRP, RR interval, PR interval, QT, QTcn and latency among different groups was performed using one-way analysis of variance and post hoc Tukey tests. Arrhythmia episodes, duration of arrhythmia, threshold dose of aconitine, and scores in the animal groups were compared using nonparametric Kruskal–Wallis and Mann–Whitney *U* tests. A P value <.05 was considered as statistically significant.

3. Results

3.1. Plasma corticosterone levels

The corticosterone levels showed no differences among the control, sham, and sleep-deprived animal groups (Table 1).

3.2. HR, BP, and PRP

The index of myocardial oxygen consumption (PRP) and HR did not show any significant differences among the different groups (Table 1). REM sleep deprivation for 72 h significantly increased the systolic and mean arterial blood pressure compared to the control group (144 ± 5 and 125 ± 3 vs 120 ± 5 and 106 ± 5 mmHg, respectively) (P < .05). Moreover, BP was significantly higher in group T72 than in groups S48 and T48 (P < .05) (Fig. 1).