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To cite this article: Phillip R. Zoladz, Anna Krivenko, Eric D. Eisenmann, Albert D. Bui, Sarah L. Seeley, Megan E. Fry, Brandon L. Johnson & Boyd R. Rorabaugh (2016): Sex-dependent effects of sleep deprivation on myocardial sensitivity to ischemic injury, Stress, DOI: 10.3109/10253890.2016.1152469

To link to this article: http://dx.doi.org/10.3109/10253890.2016.1152469

Published online: 08 Mar 2016.
Sex-dependent effects of sleep deprivation on myocardial sensitivity to ischemic injury

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Abstract

Sleep deprivation is associated with increased risk of myocardial infarction. However, it is unknown whether the effects of sleep deprivation are limited to increasing the likelihood of experiencing a myocardial infarction or if sleep deprivation also increases the extent of myocardial injury. In this study, rats were deprived of paradoxical sleep for 96h using the platform-over-water method. Control rats were subjected to the same condition except the control platform was large enough for the rats to sleep. Hearts from sleep deprived and control rats were subjected to 20 min ischemia on a Langendorff isolated heart system. Infarct size and post ischemic recovery of contractile function were unaffected by sleep deprivation in male hearts. In contrast, hearts from sleep-deprived females exhibited significantly larger infarcts than hearts from control females. Post ischemic recovery of rate pressure product and \(+dP/dT\) were significantly attenuated by sleep deprivation in female hearts, and post ischemic recovery of end diastolic pressure was significantly elevated in hearts from sleep deprived females compared to control females, indicating that post ischemic recovery of both systolic and diastolic function were worsened by sleep deprivation. These data provide evidence that sleep deprivation increases the extent of ischemia-induced injury in a sex-dependent manner.

Keywords

Sleep deprivation, heart, infarct, cardiac ischemia, ischemic injury, heart attack

Introduction

Average sleep duration in the United States has significantly decreased over the course of the past several decades (Adenekan et al., 2013). Sleep deprivation has a significant effect on cardiovascular function. Inadequate sleep is associated with impairment of the baroreceptor reflex (Almeida et al., 2014), increased risk of hypertension (Palagini et al., 2013), endothelial dysfunction (Calvin et al., 2014), and increased formation of pro-atherosclerotic foam cells in the vasculature (Carreras et al., 2014). Inadequate sleep also increases the risk of having a myocardial infarction (Hsu et al., 2015; Laugsand et al., 2011). However, it is unknown whether sleep deprivation alters the extent of myocardial injury that occurs during an ischemic insult. The goal of this study was to determine whether sleep deprivation alters myocardial sensitivity to ischemic injury and whether sleep deprivation produces similar effects in male and female ischemic hearts.

Methods

Subjects

This study utilized 19 male and 18 female Sprague-Dawley rats obtained from a breeding colony established at Ohio Northern University. All rats were 10–12 weeks of age. Starting body weights of males in the control (331 ± 18 g) and sleep deprived (344 ± 16 g) groups were similar prior to initiation of sleep deprivation. Likewise, control (289 ± 9 g) and sleep deprived (270 ± 9 g) females also had similar body weights. All experiments were approved by the Institutional Animal Care and Use Committee of Ohio Northern University.

Sleep deprivation

Rats were subjected to paradoxical sleep deprivation using the platform-over-water method (Mendelson et al., 1974). Rats in the sleep deprived group were placed on a circular platform (6 cm diameter) located 1 cm above the surface of the water (21–22°C) in a polycarbonate cage (43 × 22 × 29 cm). Muscle atonia caused the rats to fall off the platform and awaken whenever paradoxical sleep occurred. This method is a well-established model of paradoxical sleep deprivation.
KCl, 1.2 mM MgSO4, 25 mM NaHCO3, 1.2 mM KH2PO4, in ice-cold Krebs-Henseleit solution (118 mM NaCl, 4.7 mM KCl, 1.2 mM MgSO4, 25 mM NaHCO3, 1.2 mM KH2PO4, 0.5 mM Na2EDTA, 11 mM glucose, 2.5 mM CaCl2 and pH 7.4) as previously described (Rorabaugh et al., 2016). Krebs solution was perfused through the aortic cannula at a constant pressure of 80 mmHg. Contractile function of the left ventricle was measured using an intraventricular balloon connected to a pressure transducer. Data were continually recorded using a Powerlab 4SP data acquisition system (AD Instruments, Colorado Springs, CO). Hearts were equilibrated for 25 min prior to the onset of 20 min ischemia and 2 h of reperfusion. Pre-ischemic contractile function was measured immediately prior to ischemia. Post-ischemic recovery of contractile function was measured following 1 h of reperfusion. Infarct size was measured as previously described (Rorabaugh et al., 2016).

Western blots

Hearts were isolated and the atria were quickly removed. Ventricular tissue was immediately flash frozen in liquid nitrogen and stored at −80°C. ERK phosphorylation and protein kinase C-ε expression were measured in ventricular homogenates as previously described (Rorabaugh et al., 2016).

Statistical analysis

We and others have previously shown that a 20 min ischemic insult differentially effects infarct size in male and female hearts (Rorabaugh et al., 2016). Thus, data from male and female rats were analyzed separately using independent samples t tests. Alpha was set at 0.05 for all analyzes.

Results

Females

Sleep deprivation had no significant effect on pre-ischemic contractile function in female hearts (Table 1). Exposure to 20 min ischemia produced significantly larger infarcts in hearts from sleep deprived female rats compared to hearts from control animals, t(15) = 2.60, p = 0.019 (Figure 1A). Sleep deprivation also significantly attenuated post ischemic recovery of rate pressure product, t(15) = 2.20, p = 0.044 (Figure 1B), and +dP/dT, t(15) = 2.40, p = 0.028 (Figure 1C). End diastolic pressure (Figure 1D) was significantly elevated in sleep deprived females compared to control animals, t(15) = 2.80, p = 0.015, indicating that the heart was unable to adequately relax during diastole. Recovery of −dP/dT was also decreased in hearts from sleep deprived females (Figure 1E), but this did not reach statistical significance, t(15) = 2.00, p = 0.061. These data indicate that sleep deprivation increases infarct size and worsens recovery of both systolic and diastolic parameters of contractile function in female rats.

Males

In contrast to female hearts, sleep deprivation had no effect on infarct size in male hearts subjected to an ischemic insult, t(17) = 1.10, p = 0.28 (Figure 1A). Post ischemic recovery of rate pressure product, t(15) = 0.80, p = 0.47 (Figure 1B), +dP/dT, t(15) = 0.60, p = 0.56 (Figure 1C), −dP/dT, t(15) = 0.90, p = 0.37 (Figure 1E), and end diastolic pressure, t(15) = 0.60, p = 0.99 (Figure 1D), were also unaffected by sleep deprivation. These data provide evidence that sleep deprivation worsens myocardial sensitivity to ischemic injury in a sex-dependent manner.

Effect of sleep deprivation on ERK phosphorylation and PKC-ε expression

The roles of extracellular signal-regulated kinase (ERK) and PKC-ε in protecting the heart from ischemic injury are well established (Baines et al., 2002). In light of the observation that sleep deprivation worsens ischemic injury in female hearts (Figure 1), we assessed the impact of sleep deprivation on ERK phosphorylation and the expression of PKC-ε. ERK phosphorylation was significantly decreased in ventricles from sleep deprived female rats compared to control rats, t(8) = 3.70, p = 0.006 (Figure 2A). In contrast, PKC-ε expression were not monitored in this study. However, prior electrophysiological studies demonstrate that this method selectively suppresses paradoxical sleep without significantly altering slow wave sleep (Mendelson et al., 1974; Maloney et al., 2000; Mathangi et al., 2012; Sharma et al., 2015). Brain electrophysiology and markers of stress were not monitored in this study. However, prior electrophysiological studies demonstrate that this method selectively suppresses paradoxical sleep without significantly altering slow wave sleep (Mendelson et al., 1974; Maloney et al., 2000). Control rats were placed in an identical cage on a platform located 1 cm above the surface of the water. However, the platform used for control animals was large enough (13 cm diameter) for the rats to lie down and sleep. All animals had free access to food and water throughout the experiment.

Langendorff isolated heart experiments

Following 96 h in the sleep deprivation or control chambers, rats were anesthetized with pentobarbital (50 mg/kg, i.p.). Hearts were rapidly removed and cannulated while bathed in ice-cold Krebs-Henseleit solution (118 mM NaCl, 4.7 mM KCl, 1.2 mM MgSO4, 25 mM NaHCO3, 1.2 mM KH2PO4, 0.5 mM Na2EDTA, 11 mM glucose, 2.5 mM CaCl2 and pH 7.4) as previously described (Rorabaugh et al., 2016). Krebs solution was perfused through the aortic cannula at a constant pressure of 80 mmHg. Contractile function of the left ventricle was measured using an intraventricular balloon connected to a pressure transducer. Data were continually recorded using a Powerlab 4SP data acquisition system (AD Instruments, Colorado Springs, CO). Hearts were equilibrated for 25 min prior to the onset of 20 min ischemia and 2 h of reperfusion. Pre-ischemic contractile function was measured immediately prior to ischemia. Post-ischemic recovery of contractile function was measured following 1 h of reperfusion. Infarct size was measured as previously described (Rorabaugh et al., 2016).

Table 1. Parameters of pre-ischemic contractile function in female and male hearts.

<table>
<thead>
<tr>
<th></th>
<th>Rate pressure product (mmHg X bpm)</th>
<th>+dP/dT (mmHg/sec)</th>
<th>−dP/dT (mmHg/sec)</th>
<th>EDP (mmHg)</th>
<th>Coronary flow rate (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female control</td>
<td>35.938 ± 1942</td>
<td>4.921 ± 279</td>
<td>−3.158 ± 203</td>
<td>6 ± 0.4</td>
<td>12 ± 0.9</td>
</tr>
<tr>
<td>Female sleep deprived</td>
<td>32.983 ± 1962</td>
<td>5.149 ± 288</td>
<td>−3.302 ± 190</td>
<td>6 ± 0.4</td>
<td>11 ± 0.8</td>
</tr>
<tr>
<td>Male control</td>
<td>34.783 ± 1851</td>
<td>4.377 ± 179</td>
<td>−2.968 ± 150</td>
<td>6 ± 0.7</td>
<td>15 ± 0.9</td>
</tr>
<tr>
<td>Male sleep deprived</td>
<td>32.421 ± 1460</td>
<td>4.184 ± 264</td>
<td>−2.756 ± 166</td>
<td>5 ± 0.5</td>
<td>16 ± 0.9</td>
</tr>
</tbody>
</table>

Male and female data were analyzed separately by independent t test. Sleep deprivation had no significant effect (p < 0.05) on pre-ischemic parameters of contractile function in either male or female hearts.
expression was unaffected by sleep deprivation, $t(8) = 0.70$, $p = 0.47$ (Figure 2B).

**Effect on weight gain**

Control and sleep deprived males gained similar amounts of body weight over the course of the 96 h period (5 ± 2% and 3 ± 1% gain in body weight for control and sleep deprived male rats, respectively). Control and sleep deprived females also gained similar amounts of body weight (9 ± 2% and 7 ± 1% gain for control and sleep deprived rats, respectively).

**Discussion**

The primary finding of this study is that sleep deprivation increases myocardial sensitivity to ischemic injury in a sex-dependent manner. Paradoxical sleep deprivation had no significant effect on male hearts subjected to an ischemic insult. However, sleep deprivation significantly increased infarct size and worsened post ischemic recovery of contractile function in female hearts. These data extend previous work by demonstrating that inadequate sleep not only increases the likelihood of experiencing a myocardial infarction.
Hsu et al., 2015; Laugsand et al., 2011), but also increases the extent of myocardial injury. Control female hearts exhibited significantly smaller infarcts than hearts from control males. This is consistent with recent work in our own laboratory (Rorabaugh et al., 2016) as well as the work of others (Bae & Zhang, 2005). The mechanism by which female hearts are more resistant than male hearts to ischemic injury is unclear (Ostadal & Ostadal, 2014). The existence of endogenous signaling pathways that protect the heart from ischemic injury is well established. It is possible that there are sex-dependent differences in cardio-protective signaling pathways that render a greater degree of protection to female hearts (Ledvenyiova et al., 2013). Alternatively, the decreased sensitivity to ischemic injury in female hearts might be due to the cardio protective effects of estrogen or sex-dependent differences in other hormones (Murphy & Steenbergen, 2007). Regardless of the underlying cause, our data indicate that the mechanisms responsible for decreased myocardial sensitivity to ischemic injury in female hearts can be overcome by sleep deprivation.

Some studies indicate that sleep deprivation disproportionately increases the incidence of cardiovascular disease related mortality in women. Meisinger et al. (2007) reported that short sleep duration is associated with an increased incidence of myocardial infarction in women but not in men. Furthermore, data from the Whitehall II study indicates that short sleep duration and disrupted sleep both independently increase the risk of cardiovascular disease-related death in women but not in men (Rod et al., 2014). Our data suggest that this sex-dependent effect of sleep deprivation on cardiovascular-related mortality might result from the ability of sleep deprivation to increase the amount of infarcted tissue in the female heart.

The cardio protective impact of ERK signaling in the ischemic heart is well established (Baines et al., 2002). Our finding that ERK phosphorylation is suppressed in hearts from sleep deprived females is consistent with the observation that these hearts exhibit worsened ischemic injury. However, it is difficult to establish whether suppression of ERK signaling is the cause of increased myocardial sensitivity to ischemia. Further work is needed to understand the mechanism by which sleep deprivation worsens ischemic injury in the female heart.

In conclusion, this is the first study to demonstrate that sleep deprivation increases the extent of myocardial ischemic injury in a sex-dependent manner. One limitation of this study is that we measured contractile recovery in hearts that were isolated from the autonomic nervous system. This is an important limitation in light of previous work demonstrating that sleep deprivation differentially influences autonomic function in male and female rats (Matos et al., 2013). Nevertheless, these data emphasize the importance of proper sleep for minimizing myocardial ischemic injury.

**Acknowledgments**

The authors thank Joseph Lawson and Lauren Stoner for their assistance with sleep deprivation.

**Declaration of interest**

The authors report no conflicts of interest.

**References**


DOI: 10.3109/10253890.2016.1152469

Sleep deprivation worsens cardiac injury

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