Sex Differences in Blood Pressure Response to Intermittent Hypoxia in Rats

Carmen Hinojosa-Laborde, Steven W. Mifflin

Abstract—Intermittent hypoxia is used to mimic the arterial hypoxemia that occurs during sleep apnea. The present study examined the blood pressure and heart rate responses to exposure to intermittent hypoxia in male rats and in female rats before and after ovariectomy. Rats were instrumented with telemetry transmitters and blood pressure, heart rate, and activity measured during 7 days of exposure to intermittent hypoxia (3 minutes of normoxia [21% oxygen] alternating with 3 minutes 10% oxygen between 8 AM and 4 PM, remainder of day at normoxia). Blood pressure increased in males, females, and ovariectomized females in response to 7 days of intermittent hypoxia during the hours of exposure to hypoxia. Blood pressure increased less in intact females (average change in blood pressure 1.6±0.6 mm Hg, n=11) than in females studied after ovariectomy (5.1±1.1 mm Hg, n=6) or males (5.4±1.0 mm Hg, n=10). This elevated blood pressure persisted throughout the remainder of the day when the animals were not exposed to intermittent hypoxia and remained significantly attenuated in female rats. Ovariectomy abolished the protection against the elevated blood pressure response to intermittent hypoxia in females. Heart rate increased only in males, and only during the period of the day associated with intermittent hypoxia. Female rats were protected against this tachycardia independent of the ovarian hormones. These results indicate that females are protected from the hypertensive and tachycardia effects of intermittent hypoxia. (Hypertension. 2005;46[part 2]:1016-1021.)

Key Words: blood pressure ■ heart rate ■ sleep apnea syndromes

Sleep apnea is a significant health problem in the US. Whether of central or peripheral (obstructive) origin, periodic cessation of ventilation during sleep is associated with increased arterial pressure1 and elevated sympathetic nerve discharge2 during waking hours. There is also gender specificity in the incidence of sleep apnea. Premenopausal women have a lower occurrence of sleep apnea, whereas in postmenopausal women the incidence is the same as in males.3–5 Even though premenopausal women have a lower incidence of sleep apnea, they do experience sleep apnea; however, whether blood pressure increases in young women with sleep apnea is unknown.

Intermittent hypoxia (IH) is used to model the arterial hypoxemia that occurs during sleep apnea. In both animals and humans, repetitive exposures to hypoxia increase blood pressure6–9 and sympathetic nerve discharge10,11. However, all of these studies have been performed in males. In light of the evidence that premenopausal women and ovary-intact female animals exhibit reduced responses to a variety of sympatho-excitatory stimuli, we hypothesized that female rats exposed to episodes of intermittent hypoxia will be protected against the associated hypertension, and this protection will be dependent on circulating female sex hormones. The goal of the present study was to compare the cardiovascular responses to intermittent exposure in male, female, and ovariectomized female rats.

Methods

General

Experiments were performed on 11 adult female Sprague-Dawley rats and 10 adult male Sprague-Dawley rats individually housed for the duration of the study in standard environmental conditions with access to food and water ad libitum. Estrous cycles in female animals were monitored by examining the changes in vaginal epithelium obtained from daily lavage. All study protocols were approved by the Institutional Animal Care and Use Committee and conform to the National Institutes of Health Guidelines for the Care and Use of Animals in Research.

Telemetry Monitoring of Blood Pressure and Heart Rate

Blood pressure and heart rate were monitored in all rats by telemetry using the Dataquest IV system (Data Sciences Inc, St. Paul, Minn). Under gas anesthesia (isoflurane), rats were implanted with an abdominal aortic catheter attached to a TA11PA-C40 radio-telemetry transmitter. The transmitter was secured to the abdominal muscle and remained in the abdominal cavity for the duration of the experiment. Rats were placed in individual cages that were placed on top of separate RLA-3000 radio-telemetry receivers. Two weeks were allowed for recovery from surgery, after which blood pressure
and heart rate measurements obtained during a 10-second sampling period (500 Hz) were averaged and recorded every 10 minutes.

Ovariectomy Surgery

The blood pressure and heart rate effects of intermittent hypoxia in female rats were determined before and after ovariectomy (OVX) or no surgery (time controls). Immediately after the 3-week experimental protocol for intermittent hypoxia (described later), 6 female rats were anesthetized with isoflurane, and the ovaries were removed through bilateral flank incisions. After OVX, rats were allowed to stabilize for 3 weeks before a second exposure to IH. In time control experiments, the remaining 5 female rats experienced a similar delay between 2 exposures to IH with no intervening OVX surgery.

Intermittent Hypoxia Experimental Protocol

Approximately 10 to 14 days after implantation of telemetry transmitters, a standard cage containing one rat was placed in a Plexiglas chamber. A telemetry receiver was placed underneath the chamber. The oxygen (O₂) level in each chamber was monitored and regulated by a set of user-controlled timers that determined the open time of separate valves connected to room air and nitrogen, which entered the chamber via separate user-controlled flow meters. Once placed in the chamber, rats were exposed to normoxic (21% O₂) conditions (24 hours/day) and baseline values were recorded for 6 to 7 days during this control period. After the control period, the animals were exposed to a 7-day IH period. IH was generated by reducing O₂ from 21% to 10% over 1 minute, holding O₂ at 10% for 2 minutes, after which the chambers were flushed with room air to raise O₂ back to 21% over 1 minute and holding at 21% for 2 minutes before the cycle repeated itself. Each IH protocol was applied for 8 hours during the light (nocturnal) period, from 8:00 AM to 4:00 PM. The air flows and noise caused by valves opening during the IH protocols resulted in no obvious change in behavior; the rat continued to sleep quietly in the cage during the exposure to IH. After exposure to IH for 7 days, the IH was stopped and rats were again exposed to room air (24 hours/day) during a recovery period of 6 to 7 days. Therefore, the 3-week experimental protocol consisted of a control, IH, and recovery period each lasting for 1 week. Female rats were subjected to 2 rounds of the experimental protocol, i.e., before and after OVX (n=6). A 3-week stabilization period was allowed between the 2 rounds of experiments. Time control female animals were also exposed to 2 rounds of the experimental protocol separated by a 3-week period, but they were not subjected to OVX surgery (n=5).

Data Acquisition and Analysis

Mean arterial pressure (MAP), heart rate (HR), and activity were monitored during the entire control, IH, and recovery periods. Parameters were recorded and digitized using a Dataquest A.R.T. 2.2 data acquisition and analysis program (Data Sciences International) for 24 hours per day throughout the experiment. Data were sampled every 10 minutes for 10 seconds and further reduced to 1-hour averages throughout the 24-hour period. Because the exposure to IH only occurred during a portion of the day, data were further reduced to an 8-hour average covering the period of exposure to IH during the daytime or nocturnal (light with IH) period from 8 AM to 4 PM, a 5-hour period during the light period (4 PM to 9 PM, light with no IH) and a 10-hour period describing the parameters during the nighttime or active (dark) period from 9 PM to 7 AM. Telemetry transmitter offset was measured postmortem and used to adjust for any changes in MAP caused by drift in the radio signal.

The significance of effects of IH on MAP, HR, and activity during different periods of the day (light with IH, light with no IH, and dark) was determined by 2-way ANOVA with repeated measures. The results of these 2-factor ANOVAs are shown in the Table. One-way ANOVA with repeated measures analyses were used to identify differences within groups. A Holm-Sidak multiple comparison analysis was used to compare within group responses to control. To compare responses between males, females, and OVX females, the changes in MAP and HR from control values during the 3 different periods of the day were calculated and analyzed by one way

<table>
<thead>
<tr>
<th>P Calculated by 2-Way ANOVA for Repeated Measures for MAP, HR, and Activity During Light With IH, Light With No IH, and Dark Periods of the Day</th>
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<tbody>
<tr>
<td>Dependent Variable</td>
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<tr>
<td>MAP, light with IH</td>
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<td>MAP, light with no IH</td>
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<td>MAP, dark</td>
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<td>Activity, light with no IH</td>
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<td>Activity, dark</td>
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P<0.05 was considered statistically significant.

ANOVA. The significance level was P<0.05. All data were expressed as mean±SEM.

Results

IH in Males

Figure 1 illustrates that exposure of males to IH increased MAP during the 3 periods of the day (light with IH days H2 to H7 and R1 to R2; light with no IH days H1 to H7; dark days H1 to H7 and R1). HR was increased during the light with IH phase (days H2 to H7) but not during the other periods of the day. Exposure to IH did not result in any significant changes in activity during any period of the day.

IH in Intact Females

Intact female animals had normal 4-day estrous cycles during the experimental protocol. MAP varied during the estrous cycle, such that MAP tended to be lowest during the time of proestrus. Exposure of intact females to IH (Figure 2) significantly altered MAP during the period of exposure to IH (light with IH) only on 2 days (H4 and H7); however, MAP was significantly elevated during the other periods of the day for more than 5 days (P<0.05 for light with no IH on days H1 to H4, H6 to H7, and R5; P<0.05 for dark on days H2, H5 to H7, R3 and R5). HR was not significantly increased during any period of the day. Exposure to IH did not result in any significant changes in activity during any period of the day.

IH in OVX Females

Exposure of OVX females to IH (Figure 3) increased MAP during the 3 periods of the day (light with IH on days H2 to H7; light with no IH days H1 to H7; dark days H1 to H7). HR was significantly increased during the light with IH phase on day H2, but not during the light with no IH phase. During the light with no IH and dark phases, HR was significantly reduced during recovery days R1 to R7. Exposure to IH did not result in any significant changes in activity during any period of the day.

Time Control Experiments

To ensure that there were no time-dependent effects on the responses to IH, a group of intact females (n=5) was subjected to 2 sequential IH protocols using the same interval
between exposures to IH as in the OVX rats. Two-way ANOVA revealed a significant effect of IH similar to the other intact females (before OVX). However, there were no significant differences in the effects of IH on MAP or HR comparing the first to the second exposure at any period of the day, and there were no significant interactions between exposure and time (data not shown).

Sex Differences in MAP and HR

When comparing MAP and HR between males, females, and OVX by 2-way ANOVA, statistically significant differences between groups were not observed because of the variability in the control levels of MAP and HR (Table). However, statistically significant interactions between sex and time were identified. To normalize the variability between groups, the 4 days before IH were averaged to obtain a control value of MAP or HR. In addition, the last 4 days of recovery were averaged to obtain a recovery value. The changes from control for MAP (Figure 4), and HR (Figure 5) during the 7 days of exposure to IH, and the averaged recovery values were plotted as a function of time.

To facilitate comparisons between groups, the change in MAP and HR during H2 to H7 were averaged to obtain an overall response to IH for each group. The changes in MAP (Figure 4) were significantly less in intact females than in OVX females and males during light with IH (1.6±0.6, 5.1±1.0, and 5.4±1.0 mm Hg, respectively), during light with no IH (2.0±0.5, 4.5±1.3, and 4.5±0.8 mm Hg, respectively), and during the dark period (1.8±0.5, 4.6±1.0, and 4.3±0.8 mm Hg, respectively).

The changes in HR during light with IH, light with no IH, and during the dark periods were significantly greater in males compared with intact females and OVX females during each period of the day (Figure 5). There were no differences in HR response between females, and OVX females.
Discussion

Obstructive sleep apnea is a condition associated with periods of slow breathing rate, or cessation of breathing during sleep. During this time, increasing hypoxemia stimulates arterial chemoreceptors to activate the sympathetic nervous system, which leads to arousal and restoration of breathing and correction of the hypoxemia. Patients with severe sleep apnea can repeat this pattern 30 to 50 times per hour. The symptoms of sleep apnea include snoring, daytime somnolence, and hypertension. Although premenopausal females have a lower incidence of OSA than postmenopausal females or males, the incidence of hypertension in women (40 years old) was no different than in men. Sex differences in the hypertensive response to OSA have not been studied in a population 40 years old.

Sex hormones are known to play an important role in the responses and adaptations to a variety of stressors, including hypoxia. The adverse effects of exposure to continuous hypoxia are less dramatic in females than males, and sex steroids reduce many of these adverse effects. Within the central nervous system, gender and sex hormones have been shown to alter neuronal responses to hypoxia in cardiovascular-related areas, particularly in catecholaminergic structures involved in the chemoreflex pathway. Such alterations could contribute to sex-related differences in the response to hypoxia.
It should also be kept in mind that clinical studies are performed in patients with obstructive sleep apnea for unknown periods of time, but clearly long enough to result in the patient seeking treatment. The mechanisms maintaining an established hypertension in patients with sleep apnea are undergoing investigation and include an activation of the sympathetic nervous system and increased plasma levels of angiotensin II and aldosterone. However, the mechanism of the onset of hypertension is poorly understood.

In the present study, we examined alterations evoked during the first 7 days of exposure to IH to gain insight into the initial cause of the response and to insure that responses could be examined before end-organ damage that might confound interpretation. The results support our hypothesis that female rats are protected against the hypertension associated with IH.

In intact female rats, the exposure to IH increased MAP on 2 days of the exposure, and did not significantly increase HR during the light with IH period, whereas both MAP and HR were continuously elevated on days H2 to H7 during this period in males. This protection against the hypertension, but not the tachycardia was lost after removal of the female sex hormones since OVX females increased MAP similar to males, but did not respond with an increase in HR. These differences in MAP and HR between males and females were not associated with differences in animal activity. The exposure to IH occurred during the nocturnal period in rats, and the lack of change in activity during this period suggests that the exposure to IH did not result in significant arousal or motor activity.

These findings suggest that during exposure to IH, the MAP response in females is a balance of excitatory and inhibitory drives, whereas the elevated MAP response in OVX females and males during exposure to IH suggests an imbalance of excitatory and inhibitory drives favoring vasoconstriction and cardiac excitation. In males, a reduction in nitric oxide vasodilation after 7 to 35 days exposure to IH has been proposed to contribute to the elevated MAP. This IH-induced reduction in nitric oxide vasodilator function has not been studied in female rats. However, we predict that females will not exhibit this reduction in nitric oxide vasodilator function considering that estrogen promotes nitric oxide formation. We did not correlate the MAP response to IH with the estrous cycle; however, estrous cycle could contribute to the variable MAP observed during the 7-day exposure to IH (Figure 4).

IH is a widely used model of the arterial hypoxemia that accompanies sleep apnea; however, it differs from sleep apnea in that the hypoxemia in sleep apnea is associated with hypercapnia, and the hypoxemia in IH is associated with hypocapnia. Combining IH with eucapnia or hypercapnia increased the cardiovascular response to IH in male rats. Whether the response to IH in females is altered when hypocapnia is not allowed to develop is undergoing investigation. Previous studies using longer or comparable days of exposure to IH report similar changes in MAP. It is also of interest that treatment of sleep apnea typically results in a 5- to 10-mm Hg decrease in MAP.

In both male and female rats exposed to IH, MAP remained elevated during periods of the day when the rats were not exposed to hypoxia. The "carry-over" increase in MAP during nonhypoxic periods of the day was less in intact females compared with males and OVX females, suggesting that intact females are protected against the hypertensive effects of IH throughout the day, not just during the actual exposure to IH. In contrast to MAP, HR did not remain elevated during the nonhypoxic periods of the day. Therefore, IH initiates mechanisms that serve to sustain an elevated
MAP, but not HR, during periods when not exposed to hypoxia.

As previously discussed, repetitive exposures to hypoxia or sleep apnea increase MAP and sympathetic nerve discharge in animals and humans. Reactive oxygen species have been shown to enhance carotid body chemoreceptor discharge after exposure to IH, which could contribute to increased sympathetic outflow to resistance vessels. IH induced increases in MAP are also dependent on angiotensin II and endothelin. Any or all of these factors could contribute to the sustained increase in MAP throughout the day.

To summarize, this study identified sex differences in the MAP and HR responses to IH in rats that are dependent on the presence of female sex hormones. First, MAP increased in males, females, and O VX females in response to 7 days of IH during the hours of exposure to IH that persisted throughout the remainder of the day when the animals were not exposed to IH. This increase in MAP was significantly blunted in female rats and dependent on ovarian hormones. Second, HR increased only in males, and only during the period of the day associated with IH. Female rats were protected against this tachycardia independent of the ovarian hormones.

**Perspectives**

This study is the first to our knowledge to show that there is a sex difference in the cardiovascular response to intermittent hypoxia in rats, and that this difference is dependent on the presence of female sex hormones. Future studies will focus on the mechanisms by which the female sex hormone, estrogen limits the increase in MAP and HR associated with IH.

**Acknowledgments**

This work was supported by HL-66335 and a Presidential Research Enhancement Fund Award from the University of Texas Health Science Center at San Antonio.

**References**

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Hypertension. 2005;46:1016-1021; originally published online September 12, 2005;
doi: 10.1161/01.HYP.0000175477.33816.f3
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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