Chronic intermittent hypoxia alters neurotransmission from lateral paragigantocellular nucleus to parasympathetic cardiac neurons in the brain stem

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Submitted 21 April 2014; accepted in final form 14 October 2014

Dergacheva O. Chronic intermittent hypoxia alters neurotransmission from lateral paragigantocellular nucleus to parasympathetic cardiac neurons in the brain stem. J Neurophysiol 113: 380–389, 2015. First published October 15, 2014; doi:10.1152/jn.00302.2014.—Patients with sleep-related disorders, including obstructive sleep apnea (OSA), have an increased risk of cardiovascular diseases. OSA events are more severe in rapid eye movement (REM) sleep. REM sleep further increases the risk of adverse cardiovascular events by diminishing cardioprotective parasympathetic activity. The mechanisms underlying REM sleep-related reduction in parasympathetic activity likely include activation of inhibitory input to cardiac vagal neurons (CVNs) in the brain stem originating from the lateral paragigantocellular nucleus (LPGi), a nucleus that plays a role in REM sleep control. This study tests the hypothesis that chronic intermittent hypoxia and hypercapnia (CIHH), an animal model of OSA, inhibits CVNs because of exaggeration of the GABAergic pathway from the LPGi to CVNs. GABAergic neurotransmission to CVNs evoked by electrical stimulation of the LPGi was examined with whole cell patch-clamp recordings in an in vitro brain slice preparation in rats exposed to CIHH and control rats. GABAergic synaptic events were enhanced after 4-wk CIHH in both male and female rats, to a greater degree in males. Acute hypoxia and hypercapnia (H/H) reversibly diminished the LPGi-evoked GABAergic neurotransmission to CVNs. However, GABAergic synaptic events were enhanced after acute H/H in CIHH male animals. Orexin-A elicited a reversible inhibition of LPGi-evoked GABAergic currents in control animals but evoked no significant changes in CIHH male rats. In conclusion, exaggerated inhibitory neurotransmission from the LPGi to CVNs in CIHH animals would reduce cardioprotective parasympathetic activity and enhance the risk of adverse cardiovascular events.

Cardiac vagal neurons; brain stem; REM sleep; apnea; hypoxia; hypercapnia

OBSTRUCTIVE SLEEP APNEA (OSA) is a common sleep-related disorder with an incidence of approximately 24% in US adult men and 9% in US adult women (Bazzano et al. 2007; Punjabi 2008). Patients with OSA experience chronic nocturnal recurrent apneas and intermittent hypoxia, and they have an increased risk of adverse cardiovascular-related symptoms and events including sudden cardiac death, hypotension, arrhythmias, myocardial ischemia, and stroke (Kato et al. 2009; Parish and Somers 2004; Punjabi 2008). Rapid eye movement (REM) sleep further increases the health risk, as obstructive events occur for a longer duration of time and with more desaturation of oxyhemoglobin occurring during REM sleep than during non-REM sleep (Arens and Marcus 2004; Charbonneau et al. 1994; Findley et al. 1985; Goh et al. 2000; Neves et al. 2010). REM sleep independently also increases sympathetic and attenuates cardiac vagal tone and is associated with cardiac arrhythmias (Berlad et al. 1993; Valladares et al. 2008; Verrier and Josephson 2009). Accordingly, the relative risk for sudden death during REM sleep is as high as 1.2 times the risk during wakefulness (Verrier et al. 1996). Despite the evidence of these prevalent REM sleep-related adverse cardiovascular events and negative long-term consequences of OSA, the neurophysiological mechanisms that link OSA, REM sleep state, and cardiovascular abnormalities are poorly understood.

Chronic exposure to intermittent hypoxia (CIH) or hypoxia and hypercapnia (CIHH) during the nocturnal period in animals mimics the repetitive episodes of apneas that occur in humans with OSA. Both OSA patients and animals exposed to CIH have an altered balance of autonomic activity, with elevated sympathetic and reduced parasympathetic activity to the heart with resulting tachycardia and decreased baroreflex sensitivity (Bonsignore et al. 2006; Freet et al. 2013; Lin et al. 2007; Narkiewicz et al. 1998; Parati et al. 1997; Parish and Somers 2004; Reynolds et al. 2007). The results from recent animal studies suggest that the mechanisms for decreased baroreflex control of heart rate and diminished parasympathetic activity to the heart following CIHH likely involve central autonomic dysregulation and in particular altered function of parasympathetic cardiac vagal neurons (CVNs) in the nucleus ambiguus (NA).

CVNs receive neurotransmission that includes GABAergic, glycinergic, and glutamatergic inputs (Dergacheva et al. 2013; Evans et al. 2005; Neff et al. 2004). Recent work (Dergacheva et al. 2010) has found GABAergic pathway from the REM sleep-active lateral paragigantocellular nucleus (LPGi) (Sirieux et al. 2012; Verret et al. 2006) to CVNs. This pathway likely provides a neurochemical mechanism for REM sleep-related reductions in parasympathetic cardiac activity (Dergacheva et al. 2010). However, the alterations that occur with CIHH within the GABAergic pathway from the LPGi to CVNs are unknown. This study tested the hypothesis that CIHH impairs parasympathetic activity to the heart via an exaggeration of the inhibitory GABAergic pathway from the LPGi to CVNs in the NA.

Results from recent studies have demonstrated that the inhibitory neurotransmission to CVNs is very sensitive to acute hypoxia or acute hypoxia and hypercapnia (H/H) (Dergacheva et al. 2010; Neff et al. 2004). However, these central parasympathetic responses may be altered by chronic repetitive exposures to H/H. This study tested the hypothesis that CIHH alters
GABAergic neurotransmission from the LPGi to CVNs during and/or following acute H/H.

In addition to reduced parasympathetic cardiac activity (Wiklund et al. 2000), patients with OSA have significantly altered levels of the hypothalamic neuropeptide orexin-A (Igarashi et al. 2003; Liao and Yu 2005; Sakurai et al. 2005). Since orexin-A is also involved in cardiovascular regulation and, in particular, in parasympathetic control of heart rate (Ciriello et al. 2003; Dergacheva et al. 2005, 2011), it is reasonable to speculate that altered levels of orexin-A are implicated in adverse cardiovascular events associated with OSA. However, the pathophysiological mechanisms by which orexin-A may elicit the alterations in autonomic cardiovascular control associated with OSA are unknown. Accordingly, this study tested the hypothesis that orexin-A modulation of LPGi-evoked GABAergic pathway to CVNs is altered after CIHH.

MATERIALS AND METHODS

Experiments were conducted on Sprague-Dawley rats of both sexes. Animals were purchased from Hilltop Lab Animals (Scottsdale, PA) and housed in the George Washington University animal care facility under standard environmental conditions. All animal procedures were performed in compliance with the institutional guidelines at George Washington University and are in accordance with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association and the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All procedures were approved by the George Washington University Institutional Animal Care and Use Committee.

CVN labeling. Fluorescent retrograde labeling of parasympathetic CVNs was performed as described previously (Dergacheva et al. 2010, 2013). Rap pups (postnatal days 2–3) were anesthetized with isoflurane and perfused transcardially. Then the brain was carefully removed, and 300-μm-thickness slices of the medulla that contained the NA and LPGi were obtained with a vibratome. The obtained slices of the medulla that contained the NA and LPGi were obtained with a vibratome. The obtained slices of the medulla that contained the NA and LPGi were obtained with a vibratome. The obtained slices of the medulla that contained the NA and LPGi were obtained with a vibratome. The obtained slices of the medulla that contained the NA and LPGi were obtained with a vibratome.
neurons to create a summary of results for each condition. Results are presented as means ± SE and statistically compared with GraphPad Prism 5 software, using Student’s t-test, one-way repeated-measures ANOVA, or two-way ANOVA, as appropriate.

RESULTS

CIHH enhanced GABAergic neurotransmission from LP Gi to CVNs. In agreement with previously published data obtained from neonatal rats (Dergacheva et al. 2010, 2011), electrical stimulation of the LP Gi evoked GABAergic current in CVNs in the NA in adult rats (see Figs. 1–5). The peak amplitude of GABAergic IPSC was significantly enhanced by CIHH in both female rats [unexposed animals 76.0 ± 6.7 pA (n = 30) vs. CIHH animals 104.4 ± 7.4 pA (n = 31); P < 0.01, Student’s unpaired t-test; Fig. 1] and male rats [unexposed animals 85.2 ± 6.9 pA (n = 32) vs. CIHH animals 178.4 ± 18 pA (n = 25); P < 0.001, Student’s unpaired t-test; Fig. 1]. However, this CIHH-elicited exaggeration of GABAergic currents occurred to a greater degree in males compared with females. Indeed, the amplitude of LP Gi-evoked GABAergic IPSC was significantly greater (P < 0.001, Student’s unpaired t-test) in male CIHH animals [178.4 ± 18 pA (n = 25); Fig. 1] than in female CIHH animals [104.4 ± 7.4 pA (n = 31); Fig. 1], whereas there were no differences in the amplitude of LP Gi-evoked GABAergic IPSC between male and female rats not exposed to CIHH [85.2 ± 6.9 pA (n = 32) vs. 76.0 ± 6.7 pA (n = 30); P > 0.05, respectively, Student’s unpaired t-test; Fig. 1].

Effect of acute H/H on GABAergic neurotransmission from LP Gi to CVNs in unexposed and CIHH animals. Acute H/H resulted in a significant inhibition of LP Gi-evoked GABAergic neurotransmission to CVNs in all groups of animals studied (see Table 1, Fig. 2, Fig. 3). Indeed, the amplitude of GABAergic IPSC was significantly reduced during acute H/H in both unexposed female animals [from 79.8 ± 14 pA to 16.2 ± 4.1 pA (H/H at 4–5 min), then to 10.8 ± 2.0 pA (H/H at 9–10 min); P < 0.001, 1-way repeated-measures ANOVA and Dunnett’s posttest (n = 13); Table 1 and Fig. 2] and unexposed male animals [from 75.6 ± 5.0 pA to 26.4 ± 5.3 pA (H/H at 4–5 min), then to 23.8 ± 4.3 pA (H/H at 9–10 min); P < 0.001, 1-way repeated-measures ANOVA and Dunnett’s posttest (n = 16); Table 1 and Fig. 3]. Similar to unexposed animals, in CIHH rats the amplitude of GABAergic IPSC was significantly diminished during acute H/H in both females [from 105.8 ± 12 pA to 28.8 ± 7.2 pA (H/H at 4–5 min), then to 18.4 ± 3.8 pA (H/H at 9–10 min); P < 0.001, 1-way repeated-measures ANOVA and Dunnett’s posttest (n = 13); Table 1 and Fig. 2] and males [from 174.2 ± 20 pA to 81.3 ± 15 pA (H/H at 4–5 min), then to 55.0 ± 13 pA (H/H at 9–10 min); P < 0.001, 1-way repeated-measures ANOVA and Dunnett’s posttest (n = 16); Table 1 and Fig. 3].

Fig. 1. Electrical stimulation of the lateral paragigantocellular nucleus (LP Gi) evoked GABAergic inhibitory postsynaptic currents (IPSCs) in cardiac vagal neurons (CVNs) in the nucleus ambiguus (NA). This neurotransmission was significantly enhanced in both female and male rats exposed to chronic intermittent hypoxia and hypercapnia (CIHH) (Student’s t-test). Comparison between sexes revealed that the amplitudes of the GABAergic IPSCs were significantly greater in male animals exposed to CIHH than in female animals exposed to CIHH (Student’s t-test). Representative traces are shown in A, and the summary data are demonstrated in B. Arrow indicates electrical stimulation in this and all subsequent figures. Statistically significant differences: **P < 0.01, ***P < 0.001. # # # Statistically significant difference (P < 0.001) between sexes.
Table 1. Effect of acute H/H on amplitude of LPGi-evoked GABAergic IPSC in CVNs in unexposed and CIHH animals of both sexes

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>H/H (2 min)</th>
<th>H/H (5 min)</th>
<th>H/H (10 min)</th>
<th>Recovery</th>
</tr>
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<tr>
<td>Unexposed females</td>
<td>79.8 ± 14</td>
<td>52.6 ± 14</td>
<td>16.2 ± 4.1†</td>
<td>10.8 ± 2.0†</td>
<td>78.4 ± 14</td>
</tr>
<tr>
<td>CIHH females</td>
<td>105.8 ± 12</td>
<td>89.3 ± 14</td>
<td>28.8 ± 7.2†</td>
<td>18.3 ± 3.8†</td>
<td>125.9 ± 11</td>
</tr>
<tr>
<td>Unexposed males</td>
<td>75.6 ± 5.0</td>
<td>59.3 ± 7.1</td>
<td>26.4 ± 5.3†</td>
<td>23.8 ± 4.3†</td>
<td>82.7 ± 9.2</td>
</tr>
<tr>
<td>CIHH males</td>
<td>174.2 ± 20</td>
<td>148.7 ± 21</td>
<td>81.3 ± 15†‡§</td>
<td>55.0 ± 13†‡</td>
<td>233.8 ± 25*</td>
</tr>
</tbody>
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Values (in pA) are means ± SE. H/H, hypoxia and hypercapnia; LPGi, lateral paragigantocellular nucleus; CVN, cardiac vagal neuron; CIHH, chronic intermittent hypoxia and hypercapnia; IPSC, inhibitory postsynaptic current. Control vs. H/H and recovery: Statistical significance was tested by 1-way repeated-measures ANOVA and Dunnett’s posttest (*P < 0.01, †P < 0.001). Males vs. females: Statistical significance was tested by 2-way ANOVA and Bonferroni’s posttest. Statistically significant difference (§P < 0.01) was found between unexposed and CIHH males in the amount of reduction of the GABAergic IPSC by H/H (5 min).

There were no significant differences in the reduction of the amplitude of LPGi-evoked GABAergic current by acute H/H between unexposed male and female rats (P > 0.05, 2-way ANOVA, Bonferroni’s posttest). However, acute H/H at 4–5 min elicited a greater reduction in the GABAergic amplitude in CIHH female rats compared with CIHH male rats (P < 0.05, 2-way ANOVA, Bonferroni’s posttest) (Table 1). There were no significant differences in the reduction in the amplitude of LPGi-evoked GABAergic current by acute H/H between unexposed and CIHH female rats (P > 0.05, 2-way ANOVA, Bonferroni’s posttest). However, acute H/H at 4–5 min elicited a greater reduction in the GABAergic amplitude in unexposed male rats compared with CIHH male rats (P < 0.01, 2-way ANOVA, Bonferroni’s posttest; Table 1 and Fig. 2).

The GABAergic neurotransmission from the LPGi to CVNs completely recovered and was not significantly different from control levels at 19–20 min after acute H/H in both unexposed and CIHH female rats [79.8 ± 14 pA (control conditions) vs. 87.8 ± 14 pA (after H/H); P > 0.05, 1-way repeated-measures ANOVA and Dunnett’s posttest (n = 13); Table 1 and Fig. 2] and unexposed male rats [75.6 ± 5.0 pA (control conditions) vs. 82.7 ± 9.2 pA (after H/H); P > 0.05, 1-way repeated-measures ANOVA and Dunnett’s posttest (n = 16); Table 1 and Fig. 3]. Similarly, LPGi-evoked GABAergic current in CVNs under control conditions was not significantly different from that evoked at 19–20 min after acute H/H in CIHH female animals [105.8 ± 12 pA (control conditions) vs. 125.9 ± 11 pA (after H/H); P > 0.05, 1-way repeated-measures ANOVA and Dunnett’s posttest (n = 13); Table 1 and Fig. 2]. However, in CIHH male animals the amplitude of LPGi-evoked GABAergic current was significantly enhanced 19–20 min after acute H/H compared with that under control conditions [174.2 ± 20 pA (control conditions) vs. 233.8 ± 25 pA (after H/H); P < 0.01, 1-way repeated-measures ANOVA and Dunnett’s posttest (n = 13); Table 1 and Fig. 2].

Effect of orexin-A application on GABAergic neurotransmission from LPGi to CVNs in unexposed and CIHH animals. Orexin-A, applied at a concentration of 0.1 μM, elicited a significant and reversible inhibition of LPGi-evoked GABAergic current in both unexposed female animals [from 68.8 ± 9.4 pA to 37.2 ± 8.9 pA; P < 0.001, 1-way repeated-measures ANOVA and Dunnett’s posttest (n = 9); Fig. 4] and unexposed male animals [from 98.6 ± 12 pA to 46.6 ± 8.1 pA; P < 0.01, 1-way repeated-measures ANOVA and Dunnett’s posttest (n = 8); Fig. 5]. Similar to unexposed rats, orexin-A reversibly diminished LPGi-evoked GABAergic current to CVNs in CIHH female animals [from 115.4 ± 14 pA to 69.7 ± 7.9 pA; P < 0.01, 1-way repeated-measures ANOVA and Dunnett’s posttest (n = 9); Fig. 4]. However, orexin-A elicited no significant changes in LPGi-evoked GABAergic IPSC in CIHH male animals [192.4 ± 34 pA (control) vs. 165.6 ± 25 pA (orexin-A), P > 0.05, 1-way repeated-measures ANOVA and Dunnett’s posttest (n = 9); Fig. 5]. Similarly, application of orexin-A at a higher concentration of 1 μM resulted in an inhibition of GABAergic current in both unexposed and CIHH female rats [from 78.2 ± 5.8 pA to 34.1 ± 7.9 pA, P < 0.01 (n = 8) and from 96.2 ± 7.2 pA to 47.4 ± 11 pA, P < 0.05 (n = 9), respectively, 1-way repeated-measures ANOVA and Dunnett’s posttest]. Statistical significance differences: ***P < 0.001.
ANOVA and Dunnett’s posttest; Fig. 6] as well as in unexposed male rats [from 91.0 ± 22 pA to 29.8 ± 4.7 pA; \( P < 0.01 \), 1-way repeated-measures ANOVA and Dunnett’s posttest]. However, LPGi-evoked GABAergic IPSCs were decreased during acute H/H (*** \( P < 0.001 \)) and increased after acute H/H (\( ** P < 0.01 \)) in CIHH male animals (1-way repeated-measures ANOVA and Dunnett’s posttest). In addition, acute H/H (5 min) elicited a greater reduction in the GABAergic amplitude in unexposed male animals compared with CIHH male animals (2-way ANOVA and Bonferroni’s posttest, \( *** P < 0.01 \)). Representative traces are shown in A, and the summary data are demonstrated in B.

**DISCUSSION**

Major findings. There are three major findings in this study. 1) The GABAergic pathway from the LPGi to CVNs is enhanced after CIHH in both male and female animals, and to a greater extent in males. 2) Acute H/H reversibly diminishes GABAergic neurotransmission from the LPGi to CVNs in all animals studied but, however, to a lesser degree in males exposed to CIHH. In addition, in male CIHH animals LPGi-evoked GABAergic neurotransmission is enhanced after acute H/H. 3) Orexin-A diminishes GABAergic current from the LPGi to CVNs in unexposed animals of both sexes as well as in female CIHH rats. However, orexin-A elicited no significant

Fig. 3. As in female animals, acute H/H reversibly diminished LPGi-evoked GABAergic neurotransmission in CVNs in unexposed male rats (1-way repeated-measures ANOVA and Dunnett’s posttest, *** \( P < 0.001 \)). However, LPGi-evoked GABAergic IPSCs were decreased during acute H/H (*** \( P < 0.001 \)) and increased after acute H/H (** \( P < 0.01 \)) in CIHH male animals (1-way repeated-measures ANOVA and Dunnett’s posttest). In addition, acute H/H (5 min) elicited a greater reduction in the GABAergic amplitude in unexposed male animals compared with CIHH male animals (2-way ANOVA and Bonferroni’s posttest, ** \( P < 0.01 \)). Representative traces are shown in A, and the summary data are demonstrated in B.

Fig. 4. Orexin-A (0.1 μM) elicited a significant and reversible inhibition of LPGi-evoked GABAergic IPSC in both unexposed and CIHH female rats (1-way repeated-measures ANOVA and Dunnett’s posttest). Representative traces are shown in A, and the summary data are demonstrated in B. Statistically significant differences: ** \( P < 0.01 \), *** \( P < 0.001 \).
Consistent with the conclusions in previous studies (Dergacheva et al. 2010, 2011), in this study the GABAergic pathway from the LPGi to CVNs likely provides a neurophysiological mechanism for REM sleep-related reduction in parasympathetic cardiac activity. Considerable evidence indicates that LPGi neurons play an important role in REM sleep control. There is a reduction in the amount of REM sleep after lesions of the medullary reticular formation encompassing the LPGi (Holmes and Jones 1994). The LPGi contains a significant number of neurons with activity-dependent increases in c-Fos levels after REM sleep recovery from REM sleep deprivation (Verret et al. 2005, 2006). And finally, the LPGi contains neurons specifically active during REM sleep in both cats (Sakai 1988) and rats (Sirieix et al. 2012). Since REM sleep is a period of relative cardiovascular quiescence, a reduction in cardiac activity in response to REM sleep could be mediated by LPGi-cortical interactions. The present study supports this hypothesis by demonstrating that the GABAergic pathway from the LPGi to CVNs is involved in the regulation of cardiac autonomic tone during REM sleep.

**Fig. 5.** Application of orexin-A (0.1 μM) reversibly diminished GABAergic IPSCs evoked by LPGi stimulation in unexposed male rats (1-way repeated-measures ANOVA and Dunnett’s posttest). However, orexin-A (0.1 μM) did not significantly alter GABAergic IPSC in CIHH male animals (1-way repeated-measures ANOVA and Dunnett’s posttest). Representative traces are shown in A, and the summary data are demonstrated in B. Statistically significant difference: **P < 0.01.

**Fig. 6.** Similar to a concentration of 0.1 μM, application of orexin-A at a higher concentration of 1 μM resulted in a reversible inhibition of LPGi-evoked GABAergic IPSC in both unexposed and CIHH female rats (1-way repeated-measures ANOVA and Dunnett’s posttest). Representative traces are shown in A, and the summary data are demonstrated in B. Statistically significant differences: *P < 0.05, **P < 0.01.
about one-third of the neurons expressing Fos in the LPgi during REM sleep also express glycine (Boissard et al. 2002), 70% express GAD, the synthetic enzyme of GABA (Sapin et al. 2009), and many neurons in this region of the brain express both neurotransmitters (Stornetta et al. 2004), it is very likely that most if not all of the neurons specifically active during REM sleep are GABAergic and/or glycinergic neurons (Sirieix et al. 2012). The results from this study indicate there is an important GABAergic pathway from the LPgi to CVNs in the NA. During REM sleep, activation of GABAergic neurons in the LPgi may exert an inhibitory action at CVNs that maintain parasympathetic activity to the heart and likely this contributes to withdrawal of parasympathetic activity that occurs during REM sleep (Valladares et al. 2008).

**LPGi-evoked pathway to CVNs is exaggerated after CIHH.**

The neurophysiological mechanisms underlying the reduced parasympathetic activity and impaired baroreflex control of the heart observed in both OSA patients and animals exposed to CIH (Gu et al. 2007; Lin et al. 2007; Narkiewicz et al. 1998; Parati et al. 1997; Reynolds et al. 2007; Yan et al. 2008) are poorly understood. The results from this study indicate that GABAergic neurotransmission to CVNs elicited by the LPgi stimulation is significantly enhanced in both male and female animals exposed to CIH. This enhanced neurotransmission could be a result of increased sensitivity of GABAergic receptors in CVNs after CIH, as spontaneous GABAergic IPSCs in CVNs have been shown to be increased in CIH rats (Dyavanapalli et al. 2014). In addition, REM sleep-related activation of GABAergic neurons in the LPgi that likely project to CVNs could also be increased after CIH, since shortening of a REM sleep episode by hypoxic stimuli would lead to increased REM sleep drive (Hamrahi et al. 2001). This enhanced inhibitory neurotransmission to CVNs would result in CVN inhibition and reduced parasympathetic activity to the heart, which may contribute to increased risk of cardiovascular diseases. The comparison between male and female animals indicates that GABAergic synaptic currents are enhanced to a greater extent in males after CIH. Interestingly, epidemiologic studies have found strong male predominance of OSA (Lam et al. 2010; O’Connor et al. 2000; Young et al. 1993). Although the reason for higher prevalence of OSA in men is poorly understood, the sex differences in central body fat distribution and larger neck dimension as well as the influence of sex hormones have been implicated (Millman et al. 1995; O’Connor et al. 2000). In addition to male predominance of OSA, men are generally at greater risk for cardiovascular disease than age-matched women (Reckelhoff 2001). Thus greater male risk of CIH-induced adverse cardiovascular events, suggested by this study, may be a contributing factor for the overall enhanced male risk for cardiovascular diseases.

**CIHH alters responses to acute H/H in male rats.**

Acute H/H induces stress in the central nervous system and triggers important adaptive responses promoting survival (Gu et al. 2007). However, these adaptive responses could be altered by chronic exposures to repetitive episodes of H/H. Previous works have shown dramatic changes in both excitatory and inhibitory neurotransmission to CVNs elicited by acute hypoxia or H/H (Dergacheva et al. 2011; Kamendi et al. 2009; Neff et al. 2004). LPGi-evoked GABAergic current in CVNs is reversibly diminished by acute H/H in neonatal rats (Dergacheva et al. 2011). Consistently, the results from this study, conducted on adult rats, indicate that GABAergic current elicited by the LPgi stimulation is reversibly reduced by acute H/H in all animals studied. However, acute H/H elicits a lesser reduction in the GABAergic amplitude in male CIHH animals compared with female CIHH rats. Acute H/H also evokes a lesser reduction in the GABAergic amplitude in CIHH male animals compared with unexposed males. In
addition, GABAergic current is exaggerated 20 min after acute H/H in CIHH male animals. This reduced response to acute H/H and exaggerated inhibitory neurotransmission to CVNs would predict inhibition of CVN activity and impairment of cardioprotective parasympathetic activity to the heart during and after acute H/H in CIHH male animals. Similar to evoked GABAergic current, acute H/H diminishes spontaneous GABAergic IPSCs in CVNs in unexposed rats (Dyavanapalli et al. 2014). However, this inhibitory effect of H/H on spontaneous IPSCs is completely abolished in CIHH animals (Dyavanapalli et al. 2014). The different effects of acute H/H on evoked and spontaneous IPSCs in rats exposed to CIHH could point to the segregation of evoked and spontaneous signaling including accommodation of the two release forms within the same synapse, maintained via a separate pool of vesicles (Kavalali et al. 2011).

It has been demonstrated that apneic events impair the stability of parasympathetic nerve function during both REM and non-REM sleep, with greater impairment observed in REM sleep (Yamaguchi et al. 2014a, 2014b). Supporting the findings of this work, parasympathetic function during sleep has been found to be less stable in male OSA patients compared with female OSA patients (Yamaguchi et al. 2014a). The reduced parasympathetic activity to the heart, along with sympathetic hyperactivity, increases risk of tachycardia and sudden cardiac death that may occur during or after each episode of apnea, especially in men with OSA.

CIHH alters responses to orexin-A application in male rats. Orexin-A has been hypothesized to play a key role in the pathogenesis of OSA (Wang et al. 2013b). Some authors have demonstrated decreased levels of plasma orexin-A (Aksu et al. 2009; Busquets et al. 2004; Nishijima et al. 2003; Sakurai et al. 2004) while others have found increased levels of orexin-A in OSA patients (Igarashi et al. 2003; Liao and Yu 2005). The possible reason for the discrepancy of the results may include difference of the studied populations. Plasma levels of orexin-A are elevated in cases of mild sleep apnea-hypopnea syndrome, and the levels are reduced in parallel with the severity of the syndrome (Nishijima et al. 2003).

The results from this study indicate that orexin-A at both concentrations of 0.1 μM and 1 μM diminishes GABAergic neurotransmission from the LPGi to CVNs in unexposed animals as well as in female rats exposed to CIHH. These results are in contrast to previously published data indicating that orexin-A (1 μM) facilitates GABAergic current from the LPGi to CVNs in neonatal rats (Dergacheva et al. 2011). These apparent differences in responses to orexin-A application between neonatal and adult rats could be explained by developmental changes in orexinergic modulation of CVNs (Dergacheva et al. 2012, 2013). In contrast to unexposed rats of both sexes and CIHH female rats, orexin-A does not evoke any significant changes in male animals exposed to CIHH. Previous studies have demonstrated that orexin neurons are CO2 sensitive and could be activated by CO2/H+ (Williams et al. 2007); however, acute exposure to prolonged hypercapnia suppresses orexin expression in the hypothalamus (Wang et al. 2013a). It is possible that chronic exposure to repetitive episodes of H/H results in desensitization of orexin receptors in CVNs in male rats. The sex differences were found between male and female rats in both content of orexin-A in the lateral and posterior hypothalamus and expression of orexin receptors in the hypothalamus (Johren et al. 2001; Taheri et al. 1999). In addition to the sensitivity to CO2, orexin has been shown to increase neuronal viability and protect neurons against oxidative stress under conditions of chemical hypoxia (Sokolowska et al. 2014). Blunted responses to orexin-A would result in sustained GABAergic neurotransmission from the LPGi to CVNs, which would lead to decreased parasympathetic activity of CVNs in male animals exposed to CIHH. In addition, it is likely that the protective function of orexin on CVNs would be diminished in CIHH male animals.

Conclusions. This study established a central neurophysiological mechanism underlying impaired parasympathetic activity in animals exposed to CIHH. During REM sleep, activation of GABAergic neurons in the LPGi likely exerts an inhibitory action at CVNs diminishing parasympathetic activity to the heart. The inhibitory pathway from the LPGi to CVNs is significantly enhanced after CIHH exposures in both male and female rats, which would cause additional impairment of cardioprotective parasympathetic activity in CIHH animals. In addition to the exaggerated GABAergic pathway, the responses to acute H/H and orexin-A application are altered in male rats exposed to CIHH, suggesting a greater male-related vulnerability to CIHH. Exaggerated inhibitory neurotransmission from the LPGi to CVNs may play a critical role in sleep-associated high risk of tachycardia, arrhythmias, and sudden cardiac death in individuals with OSA.

GRANTS
This study was supported by J. Christian Gillin, M.D. Research Grant No. 004GN12 to O. Dergacheva.

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS
Author contributions: O.D. conception and design of research; O.D. performed experiments; O.D. analyzed data; O.D. interpreted results of experiments; O.D. prepared figures; O.D. drafted manuscript; O.D. edited and revised manuscript; O.D. approved final version of manuscript.

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