Chronic intermittent hypoxia impairs baroreflex control of heart rate but enhances heart rate responses to vagal efferent stimulation in anesthetized mice

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Submitted 14 October 2006; accepted in final form 21 March 2007

Lin M, Liu R, Gozal D, Wead WB, Chapleau MW, Wurster R, Cheng Z. Chronic intermittent hypoxia impairs baroreflex control of heart rate but enhances heart rate responses to vagal efferent stimulation in anesthetized mice. Am J Physiol Heart Circ Physiol 293: H997–H1006, 2007. First published March 23, 2007; doi:10.1152/ajpheart.01124.2006.—Chronic intermittent hypoxia (CIH) leads to increased sympathetic nerve activity and arterial hypertension. In this study, we tested the hypothesis that CIH impairs baroreflex (BR) control of heart rate (HR) in mice, and that decreased cardiac chronotropic responsiveness to vagal efferent activity contributes to such impairment. C57BL/6J mice were exposed to either room air (RA) or CIH (6-min alternations of 21% O2 and 5.7% O2, 12 h/day) for 90 days. After the treatment period, mice were anesthetized (Avertin) and arterial blood pressure (ABP) was measured from the femoral artery. Mean ABP (MABP) was significantly increased in mice exposed to CIH (98.7 ± 2.5 vs. RA: 78.9 ± 1.4 mmHg, P < 0.001). CIH increased HR significantly (584.7 ± 8.9 beats/min; RA: 518.2 ± 17.9 beats/min, P < 0.05). Sustained infusion of phenylephrine (PE) at different doses (0.1–0.4 µg/min) significantly increased MABP in both CIH and RA mice, but the ABP-mediated decreases in HR were significantly attenuated in mice exposed to CIH (P < 0.001). In contrast, decreases in HR in response to electrical stimulation of the left vagus nerve (30 µA, 2-ms pulses) were significantly enhanced in mice exposed to CIH compared with RA mice at low frequencies. We conclude that CIH elicits a sustained impairment of baroreflex control of HR in mice. The blunted BR-mediated bradycardia occurs despite enhanced cardiac chronotropic responsiveness to vagal efferent stimulation. This suggests that an afferent and/or a central defect is responsible for the baroreflex impairment following CIH.

nucleus ambiguous; obstructive sleep apnea; cardiac ganglia

OBSTRUCTIVE SLEEP APNEA (OSA) is associated with substantial morbidities involving the central nervous system and cardiovascular systems (43, 50). Chronic intermittent hypoxia (CIH), which epitomizes one of the characteristics of OSA, has been used as a useful model for OSA (18, 41). Regarding the latter, of particular physiological and clinical importance, is baroreflex control of heart rate (HR) (baroreflex sensitivity). Attenuation of baroreflex sensitivity is closely associated with severe clinical conditions, including heart failure (16, 25, 44), and is considered as an independent risk factor for cardiac failure and sudden death (22). Therefore, an increased understanding of the physiological and anatomical characteristics of CIH-associated changes of the baroreflex pathways is clearly needed for improved formulation of interventional strategies aimed at reducing the morbidity associated with OSA.

Previously, we demonstrated that baroreflex control of the HR was significantly reduced following CIH in Fisher 344 (F344) young adult rats (29). In addition, we showed that vagal cardiac motor neurons in the nucleus ambiguous projecting to cardiac ganglia play an important role in the baroreflex control of the HR (10–12). More recently, we found that postnatal CIH exposures led to altered baroreflex function in adult rats, and that the associated reduction of vagal efferent axon projections to cardiac ganglia could contribute to the long-term modification in baroreflex function (46).

Although progress has occurred in the understanding of alterations in baroreceptor reflex function following intermittent hypoxia (24, 29, 30, 42, 46, 47, 49), very little is currently known about the structural and functional changes of sensory afferent, central and efferent components of the reflex, and their molecular underpinnings. Mice are particularly useful mammalian models that are not only susceptible to genetic manipulation but can also be used in physiological, anatomical, cellular, and molecular studies (5, 6). In the present study, we hypothesized that baroreflex control of the HR was reduced following CIH, and the reduction of vagal efferent control of the heart might account for the attenuated baroreflex sensitivity. Therefore, we aimed to characterize the changes in baroreceptor sensitivity in anesthetized mice following CIH and to test whether the vagal efferent control of the heart was accordingly modified by the CIH exposure paradigm. Our primary goal was to localize the functional and structural changes of neural components within the baroreflex circuitry and thereby provide a platform for future studies aiming to elucidate the cellular and molecular mechanisms for reduced baroreflex function using transgenic approaches (48).

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MATERIALS AND METHODS

Mice (C57BL/6J, 3–4 mo, n = 36) were used. Procedures were approved by the University of Louisville and the University of Central Florida Animal Care and Use Committees and followed the guidelines established by the National Institutes of Health. Efforts were made to minimize the number of animals used and reduce suffering. Mice were exposed either to room air (RA) or intermittent hypoxia (IH) for 3 mo.

IH exposure. Animals were housed in Plexiglas chambers (30 × 20 × 20 in³; Oxycycler model A44XO; BioSpherix Instruments, Redfield, NY) in a room where light and dark cycles were set at 12-h:12-h (6:00 am to 6:00 pm). O₂ concentration in chambers was continuously measured by an O₂ analyzer and was controlled by a computerized system through a gas valve outlet. O₂ concentration in chambers were programmed and adjusted automatically. Any deviation from desired concentration was corrected by adding pure N₂ or O₂ through solenoid valves. Ambient CO₂ in the chamber was periodically monitored and maintained at 0.03% by adjusting overall chamber basal ventilation. Humidity was measured and maintained at 40–50%. Temperature was kept at 22–24°C. The IH profile consisted of alternating 21% and 5.7% O₂ every 6 min for 12-h during the light cycle and was maintained at 21% for the night period, with an overall exposure duration of 3 mo. The RA control animals were housed in room air under the same conditions as those exposed in IH in the Oxycycler chambers, except that the concentration of O₂ was maintained at 21%.

Surgical procedure. Mice were anesthetized with Avertin (0.3 g/kg ip; RA: n = 24; CIH: n = 24). Supplemental doses of anesthetics (% of the initial dose) were administered every 30 min to prevent eye blink, withdrawal reflexes, and fluctuation in arterial blood pressure. The tips of plastic silicone catheters (polyethylene-50) were tapered to ~0.3 mm in diameter, the femoral artery (left) and femoral vein (right) were exposed, and the tapered tips of two catheters were filled with heparinized saline and inserted into the femoral artery and vein, respectively. Measurement of blood pressure was through the femoral artery. Vasoactive drugs were infused into the femoral vein using a microinfusion pump. These mice were used for the measurement of baroreflex control of the HR, and for assessment of the cardiovascular responses to vagal nerve stimulation, all of which were conducted while the animals were in the anesthetized state.

To evaluate effect of Avertin anesthesia on suppression of baroreflex sensitivity, 12 conscious mice were used. These mice were anesthetized with ketamine (91.0 mg/kg ip) plus xylazine (9.1 mg/kg ip). Catheters were inserted into the left femoral artery and vein, and then they were tunneled underneath the skin and secured by sutures on the back of the mouse. The mice were then returned to their cages for recovery. The baroreflex control of the HR was tested 1 day after surgery in an isolated quiet room while the animals were in the conscious state.

Baroreflex sensitivity. The blood pressure catheter was connected to a blood pressure transducer (MII0699, AD instruments). The transducer was positioned at heart level. ABP was measured using a PowerLab Data Acquisition System (PowerLab/8 SP) and displayed on the first channel. The HR was calculated from pulse pressures in the first channel using the Ratemeter function and displayed on the second channel. Mean arterial blood pressure (MABP) and HR were recorded by averaging ABP values and pulses for 2 min. Microinfusion of phenylephrine (PE) or sodium nitroprusside (SNP) lasted 60 s for each dose. Before the end of 60 s sustained microinjection of PE or SNP at different doses (0.03, 0.05, 0.1, 0.2, 0.3, 0.4 µg/min), the HR and MABP responses had reached a plateau. The maximal HR responses relative to the HR baseline level (∆HR) to MABP change relative to the ABP baseline level (∆MABP) induced by microinfusion of PE or SNP were averaged over 5 s at the end of 60 s sustained microinjection of these vasoactive drugs for each dose. Application of the various doses of PE (or SNP) was in a random sequence. When compared with rats (46, 47), we found that more time was required to reestablish a steady baseline in the return to prestimulation values in mice. In our experiments, 45 min were used before the next microinfusion. During this interval, blood pressure and HR had already returned to their original baseline levels. We also noted that injections of PE and SNP in the same mouse frequently led to an unstable ABP in some animals. Thus PE and SNP were infused in different groups of mice during different days.

The ratio of the maximal change in HR over the change in MABP was then calculated and averaged at each dose for each drug in each animal group. The averaged ratio of HR change over MABP change (ΔHR/ΔMABP) was used as an indicator or estimate for baroreflex sensitivity. Dose-dependent curves of ∆HR/∆MABP as functions of PE and SNP concentration were plotted for RA and CIH mice. In addition, curves of ∆HR-∆MABP relationship were plotted to show the maximal HR responses induced by MABP changes after the responses had reached a steady plateau. Baroreflex sensitivity was also assessed during PE and SNP infusion at 0.4 µg/min. Changes in HR (ΔHR) were measured and averaged over 0.5 s at every 5-mmHg increase of MABP (ΔMABP = 5 mmHg). ΔHR was then plotted as a function of ΔMABP to show the transient HR responses as MABP changes.

HR and blood pressure responses to electrical stimulation of cervical vagal nerve. A cervical midline incision was performed, and the trachea was cannulated with a catheter (polyethylene-50) to facilitate ventilation in spontaneously breathing mice. The left cervical vagal nerve was carefully dissected free from surrounding structures and was cut just caudal to the nodose ganglion. After the vagal nerve was cut, the caudal cut end was placed on a pair of bipolar, platinum hook electrodes and electrically stimulated with a Grass Stimulator (S48). The stimuli [square wave pulses (30 µA; 2 ms) at 1, 3, 5, 7, 10, 15, 20, 30, and 40 Hz for 30 s] were delivered with an isolation unit (ISU 6). Maximal HR and blood pressure responses to electrical stimulation of the cervical vagal nerve were measured. The ΔHR and ΔMABP were plotted as a function of stimulation frequency.

After the onset of electrical stimulation at low frequencies, HR decreased, initially in a gradual manner and then at a more precipitous rate. We calculated the latter period, i.e., the period needed for HR responses to drop abruptly from the onset of electrical stimulation. Data are expressed as means ± SE, unless stated otherwise.

Differences between groups was determined using two-tailed t-tests (for paired and unpaired data) with statistical significance set at P < 0.05. To compare the differences of dose-dependent curves or baroreflex function curves between RA and CIH mice, two-way repeated measures ANOVA followed by Newman-Keuls post hoc tests were used.

RESULTS

IH significantly induced systemic hypertension and increased the HR. Baseline MABP and the HR were measured before drugs were applied. CIH significantly increased MABP by 19.8 mmHg (98.7 ± 2.5 mmHg, n = 6; RA: 78.9 ± 1.4 mmHg, n = 9; P < 0.01; Fig. 1A) and increased the mean HR by 12.8% (CIH: 584.7 ± 8.9 beats/min; n = 6; RA: 518.2 ± 17.9 beats/min, n = 9; P < 0.05; Fig. 1B).

CIH significantly reduced baroreflex control of the HR during PE application. To assess baroreflex sensitivity, HR responses to ABP increases induced by intravenous microinfusion of PE at different doses for 60 s were measured in RA and CIH mice. The HR decreased (ΔHR) relative to the prestimulation baseline in response to the increased ABP (∆MABP) was obtained at the level when ΔHR reached maximum along with the corresponding ∆MABP. ΔHR and ∆MABP were averaged at each PE dose within animal groups.

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For instance, MABP reached to 145.5 ± 2.9 mmHg from the baseline value of 78.9 ± 1.4 mmHg with a reflex bradycardia response of 309.9 ± 36.5 beats/min for PE at 0.4 μg/min in RA mice (n = 6). In contrast, MABP increased to 141.7 ± 3.3 mmHg from the baseline value of 98.7 ± 2.5 mmHg with a bradycardia response of 36.5 ± 5.2 beats/min for PE at 0.4 μg/min in CIH mice (n = 6). Baroreflex sensitivity, ΔHR/ΔMABP, for RA and CIH mice were 5.41 ± 0.60 and 0.84 ± 0.05 beats⋅min⁻¹⋅mmHg⁻¹, respectively (P < 0.001). Figure 2, A (RA) and B (CIH), compares HR responses to MABP changes between RA and CIH mice at three different doses of PE. Figure 2C shows the dose-dependent curves for baroreflex function (ΔHR/ΔMABP). CIH significantly decreased baroreflex sensitivity ratios at all doses above 0.1 μg/min (ANOVA, P < 0.05). Of note, baroreflex sensitivity is the measure of the HR change in response to the blood pressure change, which was here induced by vasoactive drugs, rather than to the dose of drugs. At different doses, however, the rate of the blood pressure change is different and hence the baroreflex sensitivity is different. Figure 2D compares baroreflex sensitivity using a different approach and depicts maximal HR responses (steady state) to maximal ABP changes induced by different doses of PE. ΔHR was plotted as a function of ΔMABP. The maximal ΔHR and ΔMABP responses were first averaged at different PE doses within the group, and then curves were fitted using the Boltzmann equation (32, 35): ΔHR = (A₁ − A₂)/(1 + exp[κ(ΔMABP − MABP₅₀)]) + A₂, where A₁ and A₂ are maximal and minimal bradycardic responses, κ is the slope factor, and MABP₅₀ is the MABP at one-half of the HR range. As with the previous methodological approach, CIH significantly attenuated baroreflex sensitivity (ANOVA, P < 0.01). As noted in Fig. 2D, CIH also significantly reduced the vascular response to PE injections. Figure 2E shows the dose-dependent curves such as to compare the effect of PE on maximal ΔMABP. CIH instead significantly reduced the maximal ΔMABP to PE microinfusions (ANOVA, P < 0.05), which is consistent with recent reports (20, 40).

At high doses, MABP increased very rapidly. Therefore, we measured ΔHR in response to ΔMABP during the steep rising portion of the MABP increases induced by PE infusion at 0.4 μg/min, which departs transient HR responses to ABP changes (Fig. 2F). CIH shifted the curve to the right, once again indicating a significant reduction of baroreflex sensitivity (ANOVA, P < 0.001).

Previously, we injected PE and SNP in the same rats, and data (ΔHR/ΔMABP relationship) could be fitted into regression lines (46, 47). The slope of the regression line in a group was used as an indicator for the baroreflex sensitivity for that group. In the present study, we injected PE and SNP in different mice. Therefore, we had to characterize the baroreflex sensitivity during loading and unloading separately. In addition, the HR responses (ΔHR) to very small arterial pressure (AP) changes induced by PE at low doses were minimal, and the maximal HR responses to large AP changes induced by PE at high doses approached a plateau. Therefore, we used averaged ΔHR/ΔMABP values as estimates of baroreflex sensitivity at each dose, and the data were fitted using Bolzmann equation (sigmoidal), as shown in Fig. 2D. However, for ΔMABP between 20 and 60 mmHg, data could be fitted using linear regression. Thus our current protocol for arterial pressure stimulation and data analysis was essentially identical to the approach we had previously used in rats, except that the HR responses to ΔMABP induced by PE at both the low end and high end of the response were similar to either the minimum or the maximum responses in mice.

Baroreflex control of the HR during SNP application. HR increased in response to ABP decreases induced by intravenous microinfusion of SNP at different doses for 60 s. These changes were measured in RA and CIH mice. ΔHR relative to the prestimulation baseline values in response to ABP decreases (ΔMABP) were obtained at the level at which the MABP reached a plateau. ΔHR and ΔMABP were averaged at each SNP dose within the animal group. For example, MABP decreased to a nadir of 41.3 ± 1.9 mmHg from a baseline value of 78.9 ± 1.4 mmHg with a reflex-positive chronotropic response of 11.8 ± 4.4 beats/min (n = 6) for SNP at 0.4 μg/min in RA mice. In contrast, MABP decreased to 43.8 ± 1.6 mmHg from the baseline of 98.7 ± 2.5 mmHg with increased HR responses of 6.5 ± 2.9 beats/min (n = 6) for SNP at 0.4 μg/min in CIH mice. Baroreflex sensitivity ratios (ΔHR/ΔMABP) for RA and CIH mice were 0.31 ± 0.10 and 0.11 ± 0.10 beats⋅min⁻¹⋅mmHg⁻¹, respectively. Figure 3, A and B, compared HR responses to ABP changes between RA and CIH mice at three different doses of SNP. As shown in Fig.
HR responses to blood pressure decreases induced by intravenous infusion of SNP were very small and were almost abolished by the anesthetic Avertin in both RA and CIH animals. Notwithstanding, dose-dependent curves for baroreflex sensitivity were plotted in Fig. 3. Except for at the dose of 0.3 µg/min, baroreflex sensitivity for RA and CIH mice was similar. Figure 3 compares the baroreflex sensitivity of RA and CIH mice by plotting HR against MABP, which depicts maximal HR responses (steady state). HR and MABP were averaged at different SNP doses, and curves were again fitted using the Boltzmann equation. As with previous analytical methods, the baroreflex sensitivity was not different between RA and CIH mice (ANOVA, P > 0.10).

At the high SNP dose, ABP decreased the fastest. Therefore, we measured the ΔHR in response to ΔMABP during the steep slope of MABP decreases, which depicts transient HR responses to ABP changes (Fig. 3E) at the SNP dose of 0.4 µg/min. In this context, CIH slightly reduced baroreflex sensitivity compared with RA but not significantly (ANOVA, P > 0.10).

Avertin anesthesia moderately reduced HR responses to ABP changes during PE injection and almost completely abolished the HR responses to ABP changes during SNP injection. Since the amplitude of HR responses during SNP application was quite small in both RA and CIH mice, we suspected that Avertin might have suppressed HR responses to ABP changes during SNP as well as PE infusions. We therefore measured HR responses to ABP changes induced by PE and SNP in 12 conscious (C) RA mice. Figure 4, A and B, represents typical examples of HR responses to ABP changes induced by PE and SNP infusions at three different doses (0.05, 0.1, and 0.3 µg/min), respectively. When compared with HR responses at the same PE doses in Figs. 2A and Fig. 4A, Avertin (A) dramatically reduced HR responses at the two lower doses [0.05 µg/min: −13.9 ± 9.0 beats/min (A) vs. −187.3 ± 9.0 beats/min (C); 0.1 µg/min: −46.8 ± 11.0 beats/min (A) vs. −249.4 ± 15.7 beats/min (C); P < 0.001] but not at the high PE dose [PE: 0.3 µg/min; −264.1 ± 32.6 beats/min (A) vs. −292.4 ± 16.6 beats/min (C); P > 0.05]. In contrast, Avertin almost completely abolished the HR responses to SNP infusion at all doses [0.05 µg/min: 2.2 ± 0.2 beats/min (A) vs. 199 ±
CIH accelerated the HR response to electrical stimulation of vagal nerve. CIH shortened the response time of HR to electrical stimulation of the vagus. In RA mice, HR started to decrease gradually after vagal stimulation was initiated. It then started to drop suddenly after a period and reached the peak response quickly. The period needed from stimulation onset to a sudden drop of HR was called “time to peak.” The time to peak was inversely proportional to frequency. It ranged from $2.6 \pm 0.5$ s at 7 Hz to $0.04 \pm 0.04$ s at 40 Hz in RA mice and from $0.9 \pm 0.5$ s at 7 Hz to $0.06 \pm 0.06$ s at 40 Hz in CIH mice (Fig. 6, A–C). CIH significantly accelerated the HR responses to electrical stimulation at lower frequencies ($P < 0.05$). At high frequencies (30–40 Hz), no difference was found between groups.

CIH reduced pulse pressure increase during PE application. During PE infusion, the pulse pressure was increased in both RA and CIH mice (Fig. 2, A and B). However, the pulse pressure increase in CIH mice was much lower than that in RA mice ($P < 0.01$; Fig. 7A). During SNP infusion, the pulse pressure was decreased in both RA and CIH mice (Fig. 3, A and B). No differences were found in the pulse pressure reductions between RA and CIH mice ($P > 0.10$; Fig. 7B).

CIH reduced pulse pressure during vagal stimulation. Before the left vagal stimulation, the left vagal nerve was transected. In RA mice, the pulse pressure was not changed when compared with that in vagal intact mice. In contrast, the pulse pressure was significantly decreased in CIH mice after the nerve was cut (Fig. 7C). During vagal stimulation, the pulse pressure was unchanged in RA mice, but it was significantly decreased in CIH mice (P < 0.01; Fig. 5 and Fig. 7C).  

DISCUSSION

In this study, we have shown that CIH significantly reduced baroreflex control of the HR in anesthetized mice. These data are consistent with previous findings in rats (24, 29). Since vagal efferents play a major role in the baroreflex control of the HR (12), we examined whether the HR responses to vagal efferent stimulation were attenuated following CIH and could therefore contribute to the attenuated baroreflex control of the HR. However, contrary to the original hypothesis, we found that the HR responses to vagal stimulation were actually increased at low frequencies in CIH mice, whereas at high frequencies the HR responses did not differ between RA and CIH mice. Indeed, CIH shortened the time for HR to reach maximal responses to electrical stimulation of the vagal nerve. These findings suggest that the functional connection of vagal efferent axons to the sinus node may be upregulated to compensate for the attenuated baroreflex sensitivity and that changes of other components in the baroreflex circuitry may occur to reduce baroreflex sensitivity following CIH.

Intermittent hypoxia induced hypertension and increased the HR. Fletcher et al. (13–15) reported that CIH (8 h/day for 35 days) induced systemic hypertension in rats. This observation has been subsequently confirmed by many other laboratories. Recently, Campen et al. (3) confirmed this finding in the mouse model. Less reproducible findings have been reported for HR changes following CIH. Indeed, both significant increases or no changes of the HR in rats and mice have been described after intermittent hypoxia or sleep apnea (7, 17). Tachycardia is a frequent finding in OSA patients (38). These differences may reflect species differences or alternatively may result from the different protocols of intermittent hypoxia used in the experiments. In our current study, we found ~20% increases in ABP and 12% increases in HR among CIH-exposed mice. Of note, the duration of our exposures was longer than those reported by others (3 mo). It is likely that the concurrence of hypertension and tachycardia may represent an imbalanced sympathetic and parasympathetic activity (15).

Functional and anatomical implications of augmented HR responses to vagal stimulation. In anesthetized F344 rats, we observed a reduction of baroreflex control of the HR following CIH compared with RA controls (29). More recently, decreased baroreflex sensitivity was found in conscious rats (24). Our present data show that CIH leads to reduced baroreflex
control of the HR in anesthetized mice. However, contrary to our original assumptions, the magnitude of HR was significantly increased, and the latency of the response (the time to peak) of HR upon vagal efferent stimulation was significantly decreased at low frequencies following CIH. At high frequency vagal stimulation, however, the HR responses were similar in both groups. Therefore, the control of vagal efferent axons over the heart may not be the source of the reduced baroreflex control of the HR. Since baroreflex circuitry also includes baroreceptor afferent, central interneurons, and vagal motor neurons, we hypothesize that these other components in the brain-heart circuitry might be responsible for the reduced baroreflex function induced by CIH. Interestingly, significant reductions in glutamate receptor expression (NMDA and AMPA) in the region of nucleus ambiguus occur in rats after CIH exposures, and the HR responses to microinjection of L-glutamate into the nucleus ambiguus following CIH in F344 rats are significantly reduced compared with RA control rats (29). These findings lend credence to the possibility that other relays may be affected by CIH.

To identify the anatomical basis for enhanced vagal efferent control, we recently used anterograde tracing to study vagal efferent axons and terminals in cardiac ganglia of mice (28). Our anatomical data suggest that vagal efferent synaptic-like terminals around cardiac principal neurons following CIH were significantly larger than those of RA control mice. Therefore, it is conceivable that hypertrophy of vagal efferent terminals around cardiac ganglionic neurons underlies the anatomical substrate for the enhanced control of vagal efferent axons over the heart. Based on the physiological and anatomical findings, we propose that vagal efferent axons and terminals in cardiac ganglia may undergo structural reorganization as an attempt to compensate for the overall attenuation of baroreflex function.

**CIH reduced pulse pressure.** During PE application, the pulse pressure was increased in both RA and CIH mice. The PE-induced decrease of the HR might underlie the enhanced pulse pressure, since the slower HR induced by PE would result in an increased ventricular filling, which in turn would increase the force of contraction via the intrinsic Frank-Starling mechanism. However, CIH significantly reduced the increase

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**Fig. 5.** HR and MABP responses to electrical stimulation of the left cervical vagus at 5–40 Hz (30 μA, 1 ms). A: top, RA mice. B: bottom, CIH mice. C: HR responses are plotted as the function of electrical stimulation frequency. CIH significantly enhanced HR responses to vagal stimulation at low frequencies (ANOVA; P < 0.01). HR responses at high frequencies did not significantly differ between CIH and RA mice.
of pulse pressure when compared with RA controls. As noted above, CIH reduced the increase in HR, which would have decreased the augmented filling time seen in RA mice. During SNP application, the pulse pressure was decreased equally in both RA and CIH mice. Since the reflex, positive chronotropic responses followed the decreased MABP. The reduced ventricular filling and subsequent stroke volume would then result in decreased force of contraction and eventual pulse pressure.

Sleep apnea, and more particularly, intermittent hypoxia during sleep, are associated with left ventricle hypertrophy and increased peripheral resistance of blood vessels (more stiffness and less distensibility) (23), along with increased pulmonary artery pressures. Similarly, CIH induces right and left ventricle hypertrophy in mice. At high frequency vagal stimulation, blood pressure and HR were comparable and very low in both CIH and RA mice. During this extreme challenge, the pulse

Fig. 6. CIH shorten the time to peak of the HR responses to electrical stimulation at different stimulation frequencies. The reaction period from the stimulus onset to the abrupt reduction of the HR was measured. A: top and bottom traces show the HR responses to electrical stimulation at different frequencies in RA and CIH mice, respectively. B: time to peak of HR responses was plotted as a function of frequency. CIH significantly shortened the period, indicating that CIH facilitated the vagal efferent control of the HR (ANOVA, *P < 0.05).

Fig. 7. Changes of pulse pressure. A: PE application (0.3 µg/min). B: SNP application (0.3 µg/min). C: electrical stimulation 20 Hz. PE 0.3 µg/min. *Significant difference of the group (P < 0.01) vs. the corresponding baseline. #Significant difference between the groups (P < 0.01).
pressure became much smaller in CIH mice than that of control mice. Stimulation of the vagal efferent nerve would result in a negative inotropic state of the left ventricle and produce a reduction in aortic blood pressure. Since the aorta stiffness (3) should cause high systolic pressure, we speculate that contraction of the left ventricle in CIH animals was reduced. This may diminish the pulse pressure or cardiac output in CIH rats. Consistent with such reasoning, Chen et al. (7) found that CIH attenuated left ventricle performance.

It has to be pointed out that the pulse pressure is determined by multiple factors, such as stroke volume, vascular resistance, and ventricular contractility. CIH significantly reduces the baroreflex control of the HR, increases aortic vascular stiffness, and causes ventricular hypertrophy, all of which may in turn change stroke volume, vascular resistance, and ventricular contractility. How these factors exactly interplay to determine the pulse pressure during different vasoactive drug applications following CIH is an interesting issue that deserves further investigation.

Barosensitive neurons in the brain: one or two pathways? Application of PE and SNP activates many areas in the brain that may be directly involved in mediating baroreflex control. Our data indicated that Avertin anesthesia almost completely abolished baroreflex control of the HR during SNP infusion but not during PE infusion at high doses. This observation raises the question as to whether the brain may contain two populations of neurons or pathways; namely, one that mediates depressor responses during increases in blood pressure and another that underlies pressor responses during the decrease of blood pressure. Using Fos-like immunoreactivity, Murphy et al. (36, 37) demonstrated that Fos-like proteins are differentially expressed in regionally and neurochemically specific neural populations in the brain stem following selective increases or decreases in ABP. Therefore, it is conceivable that neurons responsive to specific directional changes in ABP are segregated in brain stem regions. Recently, Henderson et al. (21) used functional magnetic resonance imaging procedures to visualize neural responses during pressor (PE) and depressor (SNP) challenges in anesthetized adult cats. Depressor challenges produced signal-intensity declines in multiple cardiovascular-related sites in the medulla, including the nucleus tractus solitarius and caudal and rostral ventrolateral medulla. Signal decreases also emerged in the cerebellar vermis, inferior olive, dorsolateral pons, and right insula. Rostral sites, such as the amygdala and hypothalamus, increased signal intensity as olive, dorsolateral pons, and right insula. Rostral sites, such as the amygdala and hypothalamus, increased signal intensity as ventrolateral medulla. Signal decreases also emerged in the cerebellar vermis, inferior olive, dorsolateral pons, and right insula. Rostral sites, such as the amygdala and hypothalamus, increased signal intensity as ventrolateral medulla.

Another aspect of this study may support the concept that anesthetic agents may have different effects on reflex pathways. Previously, Ma et al. (31) found that pentobarbital sodium selectively impaired reflex control of the HR and did not exert a major effect on reflex control of peripheral sympathetic nerve activity or vascular resistance in mice. Furthermore, the baroreflex response (sympathoexcitation) of renal sympathetic nerve activity (RSNA) during hypotension is largely attenuated, but baroreflex RSNA response (sympathoinhibition) during PE injection is rather complete in pentobarbital-anesthetized mice (31). Consistent with these observations, sympathoexcitation of RSNA during MABP decrease is dramatically blunted in inactin-anesthetized rats, but sympathoinhibition of RSNA to the increase of MABP seems similar to that in conscious rats (33, 34). Taken together, these data indicate that an anesthetic agent may differently impact on distinct aspects of reflex circuitries, and that the baroreflex circuitry of RSNA during arterial pressure decreases is apparently more sensitive to anesthesia than the pathway for baroreceptor activation of sympathoinhibition.

SUMMARY

CIH induced systemic hypertension and tachycardia and reduced baroreflex control of the HR in anesthetized mice. Despite such findings, CIH facilitated the HR response to vagal efferent stimulation, suggesting that the functional connections of vagal efferent axons to cardiac ganglia were augmented to compensate for the overall loss of baroreflex function. Therefore, other neural components in the baroreflex loop, such as baroreceptor afferents, interneurons, and vagal motor neurons in the brain stem, might be responsible for the attenuation of baroreflex sensitivity.

It has been shown that baroreflex control of HR is depressed in the patients with OSA (1, 2, 4). The mechanisms underlying the link between OSA and autonomic dysfunction are not well established (38, 39). The successful extension of previous studies from rats to anesthetized and conscious mice should provide the opportunity to simultaneously assess functional and anatomical alterations of baroreflex function within different locations in the brain-heart axis and allow for exploration of CIH-induced cellular and molecular changes in barosensitive neurons in transgenic mice (48).

ACKNOWLEDGMENTS

This study was supported by National Institutes of Health Grants HL-75034, AG-021020, HL-79636, AG-023297, and RR-019391; The Children’s Foundation Endowment for Sleep and Neurobiology Research; and the Commonwealth of Kentucky Research Challenge Trust Fund, the Institutional Fund of the University of Central Florida.

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