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Resetting of the sympathetic baroreflex is associated with the onset of hypertension during chronic intermittent hypoxia

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ABSTRACT

Chronic intermittent hypoxia (CIH) is a model of arterial hypoxemia that accompanies sleep apnea and increases resting arterial pressure (AP). We examined the effects of 7 days of exposure to CIH on arterial baroreflex control of renal sympathetic nerve activity (RSNA) and heart rate (HR) in rats. Sprague–Dawley rats (15 plus/minus 2 weeks old) were exposed to CIH (9% oxygen for 3 min every 10 min, 8 h per day) for 7 days (n = 16) while control rats (n = 18) were maintained in normoxia. Baroreflex regulation of RSNA and HR were estimated in Inactin anesthetized and artificially ventilated rats during infusions of phenylephrine and nitroprusside to manipulate AP. After exposure to CIH, resting mean AP was higher in CIH than that in control group (115 ± 7 vs. 105 ± 7, $P<0.001$). Resting HR did not differ between the two groups. Exposure to CIH shifted the AP–RSNA relationship rightward (approximately 10 mm Hg, $P<0.01$). CIH did not alter maximum gain of the baroreflex control of RSNA (−2.6 ± 0.6 vs. −2.5 ± 0.6 arbitrary units (a.u.)/mm Hg) and HR (−1.8 ± 0.6 vs. −1.8 ± 0.7 bpm/mm Hg, CIH vs. control). In addition, cardiac spontaneous baroreflex sensitivity in conscious rats (n = 8) also did not change during exposure to CIH. These results indicate that resetting of the sympathetic baroreflex control, rather than an impairment of its sensitivity, is associated with an onset of hypertension induced by CIH.

1. Introduction

Sleep apnea is an independent risk factor for developing hypertension (Peppard et al., 2000). Chronic intermittent hypoxia (CIH) has been used as a model of arterial hypoxemia that occurs during sleep apnea (Fletcher et al., 1992c). Exposure to CIH results in a sustained increase in arterial pressure (AP) that persists into the diurnal period (Fletcher et al., 1992c).

Several earlier studies (Lai et al., 2006; Gu et al., 2007; Lin et al., 2007; Yan et al., 2008; Zoccal et al., 2009; Peng et al., 2012) have examined baroreflex function after exposure to CIH. In rats (Gu et al., 2007; Yan et al., 2008) or mice (Lin et al., 2007), exposure to CIH for over 30 days induced a reduction in baroreflex control of heart rate (HR) that was associated with hypertension. Fletcher et al. (1999) examined change of AP over a 35-day period during exposure to CIH in conscious rats and found that the elevation of AP at 7 days of exposure was not different than the AP observed at 35 days of exposure, indicating that the sustained increase in AP induced by CIH is maximal after 7 days of exposure. However, baroreflex control of sympathetic nerve activity (SNA) after 7 days of exposure to CIH remains unclear, although the AP elevation induced by CIH results from sympathetic activation (Fletcher et al., 1992b).

In previous studies, exposure to CIH for 10 days increased baroreflex sensitivity for HR despite an increasing AP (Zoccal et al., 2009). Lai et al. (2006) examined changes over time in AP and spontaneous baroreflex sensitivity (SBRS) during CIH exposure. They demonstrated that AP was elevated in rats after exposure to CIH for 5 days, while a reduction in spontaneous baroreflex sensitivity was not observed until the 17th day of CIH. These findings suggest that an onset of hypertension induced by CIH may not be secondary to reductions in the baroreflex sensitivity. Accordingly, we hypothesized that 7 days of exposure to CIH which results in a sustained increase in AP would reset baroreflex control of SNA rather than reduce its sensitivity. To test our hypothesis, we examined baroreflex stimulus–response curves of SNA in anesthetized rats after CIH exposure for 7 days. We also examined AP and cardiac SBRS before, during and after 7 days of exposure to CIH in conscious rats.

2. Materials and methods

2.1. General

Experiments were performed using male adult Sprague–Dawley rats. All rats were given at least 1 wk to acclimate before being used for any procedures. The rats were randomly assigned either a CIH group (n = 8, 14 ± 2 weeks old, 463 ± 12 g) or a control group (n = 7, 14 ± 2 weeks old, 498 ± 25 g, at the end of experiment) in conscious animal experiments. In anesthetized animal experiments, a separate group of rats from those used in the conscious animal experiments

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were randomly assigned to either a CIH group (n = 16, 15 ± 1 weeks old, 450 ± 11 g) or a control group (n = 18, 15 ± 1 weeks old, 451 ± 14 g). The Institutional Animal Care and Use Committee of the University of North Texas Health Science Center approved all experimental protocols.

2.2. Chronic intermittent hypoxia exposure

CIH rats were housed in hypoxia chambers with ad libitum food and water access as previously described (Zhang et al., 2008). O_2 concentration in the chambers was controlled using 100% N_2 and 100% O_2 by a computerized system (Oxycycler, Biospherix, NY, USA). The intermittent hypoxia was set to cycle at 9% O_2 for 6 min and 21% O_2 for 4 min. In this setting, O_2 concentration of 9% was maintained for approximately 3 min in each hypoxia cycle (Fig. 1). Exposure to CIH occurred for 8 h during the light period (8:00 to 16:00) for 7 days. The control rats were housed in normoxic conditions. In experiments of anesthetized rats, the CIH rats were taken out of the intermittent hypoxia chamber in the morning following the last day of exposure. Therefore, the baroreflex curves were obtained at 20–22 h after the last exposure to intermittent hypoxia.

2.3. Assessment of AP, HR, SBRS and respiratory frequency in conscious rats

A pressure transmitter (TA11PA-C40 Implant: DSI, MN, USA) was implanted in anesthetized rats (2% Isoflurane). Seven days after implant surgery, mean AP (MAP), HR and respiratory frequency were recorded for 10 s every 10 min (144 times a day). In addition, AP was also recorded in a continuous manner for 1 h every 12 h (at 0:00 and 12:00) for estimation of SBRS.

2.4. Assessment of baroreflex function curves in anesthetized rats

2.4.1. Surgical preparations

The rats were anesthetized with the long-acting rodent anesthetic thiobutabarbital sodium (Inactin, SIGMA) at the initial dose of 110 mg/kg ip. Supplemental anesthetic was given in doses of 10 mg/kg ip as required to maintain a surgical plane of anesthesia (i.e., an absence of withdrawal to pinch of the hind paw and no evidence of fluctuations in AP in response to surgical manipulation or pinch of the hind paw following paralysis). Gallamine triethiodide (5 mg/kg/h) was infused intravenously to induce paralysis. Body temperature was maintained at approximately 38 °C using a heating pad. The animals were intubated through a tracheotomy and mechanically ventilated with oxygen-enriched room air. A catheter was inserted into the abdominal aorta via the femoral artery and was used for the measurement of AP. HR was calculated from the AP waveform. Three catheters were inserted into the femoral vein for the infusion of drugs.

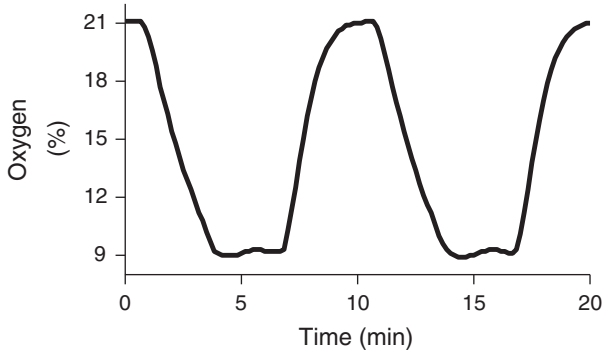

Fig. 1. Percentage of oxygen in the chronic intermittent hypoxia (CIH) chambers. Oxygen was maintained at 9% for 3 min in each hypoxia cycle. Exposure to CIH occurred for 8 h during the light period (8:00 to 16:00) for 7 days.

We exposed the left renal sympathetic nerve retroperitoneally and attached a pair of Teflon-coated stainless steel wires to record renal SNA (RSNA). The nerve and electrodes were secured with silicone glue (Kwik-Sil, World Precision Instruments, FL, USA). The nerve signal was amplified (×20,000–50,000) with the band-pass filters set between 100 and 3000 Hz. The filtered signal was full-wave rectified and integrated using a time constant of 5 ms to quantify the nerve activity.

2.4.2. Protocols

The experimental protocol was initiated at least 1 h after completion of the surgical procedures. After baseline AP and HR were obtained for 5 min, baroreflex function curves of RSNA and HR were determined during the infusion of phenylephrine HCI (100 μg/ml) to increase AP and sodium nitroprusside (100 μg/ml) to decrease AP. The rate of infusion was adjusted to produce continuous changes in AP at a rate of 1 mm Hg/s (Fig. 2). The order of administration of phenylephrine and nitroprusside was randomized.

2.5. Data analysis

2.5.1. MAP, HR, SBRS and respiratory frequency in conscious rats

To determine changes in MAP, HR, SBRS and respiratory frequency, we recorded AP at a sampling rate of 250 Hz. HR was calculated from pulse interval obtained from the AP waveform, respiratory frequency was estimated by fluctuation of the AP (Dataquest, DSI, MN, USA). MAP, HR and respiratory frequency were averaged in light period during CIH (8:00 to 16:00) and in dark period (19:00 to 7:00).

In the same group of animals, SBRS was evaluated by sequence analysis technique using the freely available software, HemoLab (Zoccal et al., 2009). SBRS was assessed by averaged slope of the linear regression between systolic AP and subsequent pulse interval pairs. A slope was obtained from progressive increases and decreases of four or more values of systolic AP that paralleled changes in pulse interval (3 beats of delay) with linear correlation higher than 0.8.

2.5.2. Baroreflex function curves in anesthetized rats

We recorded AP, RSNA and HR at a sampling rate of 1000 Hz in Inactin anesthetized rats. Because the absolute magnitude of RSNA depended on recording conditions, RSNA was presented in arbitrary unit (a.u.). Nerve activity after sacrifice was used to determine "0 a.u.". To determine "100 a.u." of RSNA, we used two different approaches as in our previous study of baroreflex regulation of RSNA in hypertensive rats (Vitela et al., 2005). In the first approach, the baseline RSNA in each animal was used to determine "100 a.u.". In the second approach, the maximum RSNA observed during the decrease in AP produced by the infusion of nitroprusside was used to determine "100 a.u.". The values for RSNA and HR during the reflex function curves were averaged into 5-mm Hg bins of AP. The baroreflex function curves of the RSNA control (AP–SNA relationship) and the HR control (AP–HR relationship) were described using a four-parameter logistic function as follows (Kent et al., 1972):

$$y = \frac{P_1}{1 + \exp[P_2(x - P_3)]} + P_4 \quad (1)$$

where x and y denote the input (AP) and output (RSNA or HR), respectively; P_1 is the response range of output; P_2 is the slope coefficient; P_3 is the midpoint pressure of input; and P_4 is the minimum value of output. The maximum gain (G_{max}) is $-P_1P_2/4$.

2.6. Statistical analysis

All data are presented as mean ± SE. The effects of CIH on MAP, HR, SBRS and respiratory frequency in conscious rats were tested by two-way ANOVA with repeated measurements. In the case of a