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Impaired control of renal sympathetic nerve activity following neonatal intermittent hypoxia in rats

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Abstract

Apneas and recurring oxygen desaturations can occur in preterm infants and young children. To investigate long-term effects of neonatal intermittent hypoxia on baroreflex control of sympathetic nerve activity, we studied 5–7-month-old (adult) Sprague–Dawley rats exposed to chronic intermittent hypoxia (CIH, $n=9$; 8% O_2 for 90 s alternating with 90 s 21% O_2, 12 h/day) for their first 30 postnatal days or controls exposed to normoxia (C, $n=9$). In adult CIH and C rats, baseline heart rate, mean arterial pressure, and plasma concentration of epinephrine and norepinephrine were similar. Baroreflex sensitivity was evaluated in anesthetized rats by changes in renal sympathetic nerve activity (RSNA) in response to i.v. infusions of phenylephrine (PE,1.5 μg/min/100 g) and sodium nitroprusside (SNP, 1.5 μg/min/100 g). Acute intermittent hypoxia (AIH, 18 min) induced elevations in RSNA by over 30% of baseline about three times more often in the CIH group than in the C group. After AIH, the gain of the baroreflex sympatho-excitatory response increased by approximately two times in C and did not change in CIH rats. The gain of sympatho-inhibitory responses to SNP at the maximum decrease in MAP was similar in the two groups in normoxia and was not affected by AIH. We conclude that postnatal intermittent hypoxia causes long-lasting impairment in chemoreceptor and baroreceptor control of renal nerve activity.

Keywords: Neonatal intermittent hypoxia; Sympathetic; Baroreflex; Chemoreflex

In recent years, a number of studies have addressed intermittent hypoxia (IH) as a factor in the mechanisms of neural plasticity [1,3,4,15]. Effects of chronic intermittent hypoxia (CIH) in the form of long-term facilitation (LTF) of neural activity have been observed in adult rats in relation to phrenic [1], hypoglossal [5], sympathetic nerve activity [3,15], and chemoreceptor sensory discharge in adult and neonatal rats [14,15]. Alterations in the corresponding central networks can result in sympathetic overactivity and hypertension [13,17]. Although IH is a hallmark of recurring apneas during sleep, the pathogenesis of sleep apnea syndrome appears more complex.

In a model of sleep apnea in adult rats that involves episodic hypoxia, blood pressure was elevated in a similar fashion by a 35-day exposure to IH and has been prevented by carotid body denervation, or pharmacological sympathectomy [4]. During early development, normoxia is required for normal maturation of chemoreceptor responses [2]. Increased vulnerability to IH during the early postnatal period [2,6,12,14,18] and IH-induced plasticity [3,15] could impose long-term modifications in cardiovascular control. However, these effects of IH are not well known.

Rats exposed to IH during postnatal days P1-P30, as adults, had attenuated baroreflex control of heart rate (HR) and reduced vagal projections to atrial ganglia, revealing long-term deficits in parasympathetic regulatory mechanisms [18]. In the present study, we tested the hypothesis that postnatal IH will induce sympathetic overactivity and modify sympathetic baroreflex control in adult rats. The sympathetic chemoreflex and baroreflex were examined in adult rats that had been exposed to postnatal IH.

The experimental procedures were in compliance with the National Institutes of Health guidelines for the use of laboratory animals and approved by the Institutional Animal Care and Use Committee. Newborn male pups delivered by time pregnant Sprague–Dawley rats (Charles River Laboratories) were

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exposed to chronic intermittent hypoxia (CIH group, $n = 9$) or to normoxia (room air controls, C group, $n = 9$) for postnatal days 1–30. Rats were exposed to 30 day chronic IH while housed in Plexiglas chambers operating under a 12 h light–dark cycle (Oxycycler model A44XO, Biospherix, Redfield, NY, USA). Computer-generated IH profiles simulated O_2 levels in obstructive sleep apnea syndrome (OSA) and were 8% O_2 (90 s) alternating with 21% O_2 (90 s), 12 h/day during daylight or sleep hours in rats and 21% O_2 during the remaining 12 h. Ambient CO_2 was 0.03%. Experiments were conducted when rats were 5–7 months old. Body weights of CIH and C rats (499.6 ± 25.4 g and 474.8 ± 11.7 g, respectively) were not significantly different.

The following protocols were completed: (1) assessment of baseline renal nerve activity, mean arterial pressure (MAP), heart rate and plasma concentration of norepinephrine (NEpi) and epinephrine (Epi) in normoxia; (2) evaluation of the sympathetic chemoreflex by the effect of 18 min acute intermittent hypoxia (AIH) on baseline sympathetic activity (during the 6th hypoxia episode of AIH and in normoxia after AIH); and (3) evaluation of the sympathetic baroflex sensitivity (BRS) to increases and decreases in MAP caused by phenylephrine (PE) and sodium nitroprusside (SNP).

Rats were anesthetized (sodium pentobarbital, 50 mg/kg i.p.) and breathed spontaneously via a cannula placed into the trachea. The corneal reflex and hind paw pinch reflex were periodically evaluated to assess the level of anesthesia and supplemental doses (10 mg/kg) were given as needed. Catheters (PE50) were placed into the femoral vein and artery. The arterial catheter was connected to a pressure transducer (model P23 db, Statham Laboratories, Inc., Hato Rey, Puerto Rico) to monitor arterial blood pressure and heart (pulse) rate via a tachometer (Grass model 7P4H). PE and SNP were infused into the femoral vein by syringe pump (sp101i, Stoelting Co) at 1.5 μg/min/100 g for 1.5–2 min to achieve MAP and renal sympathetic nerve activity (RSNA) plateaus. Acute exposures to IH lasted 18 min and included alternating six 90 s episodes of 10% and 21% O_2.

The right or left renal nerve was cut and RSNA was recorded by placing the central end on bipolar platinum electrodes connected to a high impedance probe (Grass Instruments). Nerve signals were amplified (differential preamplifier model 511, Grass Instruments), filtered (30–3000 Hz), and integrated per 1 s (1/RC = 50 ms; Grass P10F cumulative integrator). Signals were displayed on a digital storage oscilloscope (Gould, model 1425, Essex, England) and monitored by a loudspeaker. Signals representing MAP and RSNA were recorded by a polygraph (Grass, model 7). Noise during RSNA recording was estimated by silencing the nerve activity (PE infusions) and verified by crushing the nerve at the end of experiments. For analysis of RSNA, the noise was subtracted from the signal. To calculate chemoreflex effects, RSNA was averaged over 20 s intervals during baseline, the 6th hypoxia episode of AIH, and the return to normoxia after AIH. RSNA was compared to the baseline before AIH. To avoid sympatho-excitatory effects of baroreceptors, nerve responses during post-AIH normoxia were analyzed in records where MAP after AIH was higher or equal to baseline MAP. To quantify RSNA due to baroreflexes, RSNA activity was averaged over 20 s intervals during baseline and 10–20 s intervals during changes in blood pressure caused by PE and SNP (see below). Changes in RSNA are presented as a percent of baseline activity.

To evaluate BRS, PE and SNP were infused three to five times during normoxia at 5–10 min intervals before AIH exposures and one to two times following AIH exposures. Sympathetic baroreflex gain was analyzed during the early phase of the reflex when blood pressure changed rapidly and when it reached a plateau. BRS was assessed by measuring the changes from baseline RSNA at 25 mmHg steps (MAP ± 25 and MAP ± 50 mmHg) in response to PE or SNP. Changes in RSNA were averaged for each animal (and group), plotted against changes in MAP, and fitted by a regression line [10]. The slope of the line (BRS, ΔRSNA/mmHg) during normoxia, was compared in the CIH and C groups before and after AIH. During the baroreflex plateau, BRS was calculated as the ratio ΔRSNA/ΔMAP (changes from baseline to plateau) during PE and SNP infusion. Data averaged for CIH and C groups was compared.

Epinephrine and norepinephrine plasma concentrations were measured in duplicate in 300 μl samples from adult anesthetized rats (6/group) using a CatCombi ELISA kit (Cat. -No: RE 592 42, IBL, Hamburg). Samples were analyzed in the same assay and the intra assay coefficients of variation were 7.92% and 6.92% for NEpi and Epi, respectively. NEpi and Epi ELISA sensitivity was 20 pg/ml and 10 pg/ml, respectively. Data are presented as mean ± S.E.M. Regression analysis was used to calculate sympathetic baroreflex gain in each animal and group. Responses of the CIH and C groups were compared by using an ANOVA or paired t-test. Statistical significance was indicated by $P < 0.05$.

In 5–7 month old C and CIH rats, resting cardiovascular variables, catecholamine concentrations (Table 1), and baseline renal nerve activity were similar. The nine CIH rats were exposed to AIH 20 times (2–3 AIH per rat) and in 17 of the 20 exposures (85%), baseline RSNA increased after AIH (Fig. 1). The nine control rats were exposed to AIH 17 times (one to two AIH exposures per rat), and baseline RSNA increased in five (29.4%) exposures. Thus, the acute increase in baseline RSNA occurred about three times more frequently in CIH rats.

During AIH, baseline RSNA in response to hypoxia (the 6-th episode) increased more in CIH rats than in controls ($97.7 \pm 12.8\%$, $n = 7$ and $43.4 \pm 11.6\%$, $n = 7$, respectively; $P = 0.015$). Following AIH, baseline activity remained elevated during normoxia ($P < 0.05$), but was not significantly different

Table 1
Cardiovascular variables and catecholamine concentrations in adult CIH and Control rats

Groups	MAP mmHg ($n = 9$)	HR bpm ($n = 9$)	Epi pg/ml ($n = 6$)	NEpi pg/ml ($n = 6$)
Control	111 ± 5	333 ± 22	90.3 ± 9.7	305.3 ± 12.9
CIH	109 ± 2	325 ± 22	102.3 ± 5.0	305.5 ± 17.7

MAP; mean arterial pressure; HR, heart rate; Epi, epinephrine; NEpi, norepinephrine; CIH, rats exposed to chronic intermittent hypoxia during postnatal days 1–30; Control, rats reared in normoxia.