Published in final edited form as:

Respir Physiol Neurobiol. 2015 January 15; 206: 53–60. doi:10.1016/j.resp.2014.11.011.

Respiratory modulation of sympathetic activity is attenuated in adult rats conditioned with chronic hypobaric hypoxia

Yee-Hsee Hsieh¹, Frank J. Jacono^{1,2}, Ruth E. Siegel^{3,4}, and Thomas E. Dick^{1,4}

¹Division of Pulmonary, Critical Care and Sleep Medicine, Department of Medicine, Case Western Reserve University, Cleveland, Ohio, United States

²Division of Pulmonary, Critical Care and Sleep Medicine, Louis Stokes VA Medical Center, Cleveland, Ohio, United States

³Department of Pharmacology, Case Western Reserve University, Cleveland, Ohio, United States

⁴Department of Neurosciences, Case Western Reserve University, Cleveland, Ohio, United States

Abstract

Respiratory modulation of sympathetic nerve activity (SNA) depends on numerous factors including prior experience. Exposing *naïve* adult, rats (Sprague-Dawley) to acute intermittent hypoxia (AIH) enhances respiratory-modulation of splanchnic SNA (sSNA); whereas conditioning to chronic hypobaric hypoxia (CHH) attenuated modulation. We hypothesized that AIH in CHH rats would restore respiratory modulation of SNA. In anesthetized, CHH-conditioned (0.5 atm, 2 wks) rats (n=16), we recorded phrenic and sSNA before during and after AIH (8% O₂ for 45s every 5min for 1h). At baseline, sSNA was not modulated with respiration. The sSNA was not recruited during a single brief exposure of hypoxia nor after 10 repetitive exposures. Further, the sSNA chemoresponse was not restored 1h after completing AIH. Thus, CHH-conditioning blocked the short-term plasticity expressed in sympatho-respiratory efferent activities and this was associated with reduced respiratory modulation of sympathetic activity and with attenuation of the sympatho-respiratory chemoresponse.

© 2014 Elsevier B.V. All rights reserved.

Corresponding author: Yee-Hsee Hsieh, PhD, Division of Pulmonary, Critical Care and Sleep Medicine, Department of Medicine, Case Western Reserve University, 10900 Euclid Avenue, Cleveland, OH 44106-1714, Tel: (216) 368-5287, yhsieh13@gmail.com.

Disclosures: No conflict of interest, financial or otherwise, are declared by the author(s)

Author contributions: Y-H.H., R.E.S., and T.E.D., conception and design of research; Y-H.H., and T.E.D., performed experiments; Y-H.H. and T.E.D., analyzed data; Y-H.H., F.J.J., and T.E.D. interpreted results of experiments; Y-H.H. and T.E.D., prepared figures; Y-H.H., F.J.J., R.E.S., and T.E.D., drafted manuscript; Y-H.H., F.J.J., and T.E.D., edited and revised manuscript; Y-H.H., F.J.J., R.E.S., and T.E.D., approved final version of the manuscript.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Keywords

Neural control of respiration; Neural control of sympathetic activity; Hypoxia; Adaptation to hypoxia

1. Introduction

The cardiovascular-respiratory control systems are coupled and this coupling is dynamic, varying in health and disease (Garcia *et al.*, 2013). For instance, cardiorespiratory coupling is increased in elite athletes (Aubert *et al.*, 2003) whereas it is decreased in sepsis (Dick *et al.*, 2012). Conditioning to hypoxia can evoke increases or decreases in sympatho-respiratory coupling depending on whether the exposure is intermittent or sustained. Chronic intermittent hypoxia enhances respiratory modulation of splanchnic sympathetic nerve activity (sSNA) (Zoccal *et al.*, 2009) with additional activity recruited specifically in late expiration (Abdala *et al.*, 2009). This type of sympatho-respiratory coupling is associated with hypertension (Zoccal *et al.*, 2009; Abdala *et al.*, 2009). In contrast, sustained or hypobaric hypoxia has not been associated with hypertension nor enhanced respiratory modulation (Ilyinsky *et al.*, 2003; Hsieh *et al.*, 2004; Ilyinsky & Mifflin, 2005). However recently, Moraes and colleagues (Moraes *et al.*, 2014) reported that after 24h of sustained hypoxia, increased sympatho-respiratory coupling was associated with an increase in blood pressure. Our general working hypothesis is that mechanisms of plasticity for the control of the respiratory system also effect SNA. It is in this context that we address whether chronic hypobaric hypoxic (CHH) exposure impacts both sympathetic and phrenic plasticity.

1.1 Response to Hypoxia

In naïve adult male Sprague-Dawley rats, the hypoxic ventilatory response (HVR) has a stereotypic pattern, which consists of an asymptotic increase in phrenic nerve activity (PNA) burst amplitude whereas burst frequency acutely increases then decreases (Powell *et al.*, 1998). Immediately after hypoxia, PNA burst amplitude gradually returns to baseline whereas frequency decreases below baseline and recovers slowly. This period is referred to as post-hypoxic frequency decline (PHFD) (Coles & Dick, 1996). The HVR and PHFD depend on the duration and intensity of the exposure, the genetic background, the age of the animals as well as the prior exposure to hypoxia (Aaron & Powell, 1993; Dwinell & Powell, 1999; Ilyinsky *et al.*, 2003; Hsieh *et al.*, 2004; Ilyinsky & Mifflin, 2005). Conditioning rats with CIH increases baseline sympatho-respiratory coupling and accentuates the HVR (Baker & Mitchell, 2000; Fuller *et al.*, 2000; Xing & Pilowsky, 2010; Xing *et al.*, 2013). In contrast CHH conditioning attenuates both HVR and PHFD (Ilyinsky *et al.*, 2003; Hsieh *et al.*, 2004; Ilyinsky & Mifflin, 2005).

The sympathetic nerve activity has tonic and respiratory modulated components. During hypoxia respiratory modulation dominates and sSNA is activated during expiration and quiescent during inspiration. Further, sSNA is quiescent immediately after hypoxia. During PHFD, sSNA remains respiratory-modulated as it returns to baseline levels (Dick *et al.*, 2004).

1.2 Plasticity Evoked by Acute Intermittent Hypoxia

In *naïve* rats, acute intermittent hypoxia (AIH) evokes sustained increases in both PNA and sSNA (Dick *et al.*, 2007), similar to long-term facilitation (LTF) as defined for respiratory motor activity (Millhorn *et al.*, 1980b, a; Baker & Mitchell, 2000; Fuller *et al.*, 2000; Baker *et al.*, 2001; Dick *et al.*, 2007). CHH conditioning blocked LTF in PNA and sSNA (Hsieh *et al.*, 2008). However, LTF in sSNA can occur in the absence of respiratory LTF (Xing & Pilowsky, 2010) and AIH facilitates motor function in general (*i.e.*, the recovery of function in spinal cord injured (hemisection) rats (Fuller *et al.*, 2003; Golder & Mitchell, 2005; Fuller *et al.*, 2006)).

Both PHFD and LTF are forms of activity-dependent plasticity (Poon & Siniaia, 2000) and are abolished by conditioning with hypoxia (Ilyinsky *et al.*, 2003; Hsieh *et al.*, 2004; Ilyinsky & Mifflin, 2005; Hsieh *et al.*, 2008). The concept that the plasticity of a system is itself 'plastic' is termed *metaplasticity* (Abraham & Bear, 1996; Baker & Mitchell, 2000; Fuller *et al.*, 2000; Poon & Siniaia, 2000; Song & Poon, 2004). The effect of CHH-conditioning on the sympathetic chemoreflex is poorly understood. We hypothesized that CHH conditioning would unmask the tonic sympathetic chemoreflex because the HVR is blunted. Thus, we indirectly tested the hypothesis that AIH uses a common mechanism to evoke LTF in sSNA and PNA; in that AIH does not evoke LTF in tonic component of sSNA via the sympathetic chemoreflex in the absence of a strong HVR.

2. Methods

2.1 Experimental details

Surgical procedures and experimental protocols followed NIH guidelines and were approved by the Institutional Animal Care and Use Committee at Case Western Reserve University. The surgical and electrophysiologic methods for determining the hypoxic response was performed as previously reported (Dick *et al.*, 2004; Hsieh *et al.*, 2004; Dick *et al.*, 2007; Hsieh *et al.*, 2008). In brief, adult male Sprague-Dawley (Zivic Miller) rats ($n = 16$) were conditioned for 14 days to sustained hypoxia in a hypobaric chamber (0.5 atm); with ambient O_2 equal to 21% of the atmospheric gas, the inspired O_2 content is approximately that of 10% O_2 at 1 Torr. Throughout the 14 days, the chamber was opened every 2 days (< 5 min) for animal husbandry.

A small set of *naïve* rats ($n=3$) were housed in a neighboring hypobaric chamber but remained at atmospheric pressure. Data from these rats were not different than those from experiments performed previously on *naïve* rats (Dick *et al.*, 2004; Hsieh *et al.*, 2008), so the *naïve* data set incorporated the previously obtained data ($n=12$). The representative traces for the *naïve* rats are from previously unreported data.

Surgery and experiments were performed immediately following the conditioning period. Rats were anesthetized with equithesin (30 and 133 mg/kg sodium pentobarbital and chloral hydrate, respectively; administered intraperitoneally) and the initial and surgical anesthetic level was tested by paw pinch and evaluating withdrawal reflex. Once neuro-muscular blockade was administered, anesthetic effects were evaluated by observing the sympatho-

respiratory neural response after painful paw pinch. If additional anesthetic was required, the 0.1 the original dose was delivered intravenously (iv).

The femoral artery was cannulated to measure blood pressure; the femoral vein, to administer pharmacological agents; and the trachea, to ventilate the animals. The cervical vagi were transected bilaterally and the animal was placed in a stereotaxic apparatus. The left phrenic and splanchnic sympathetic nerves were isolated, transected, and mounted on bipolar electrodes for recording. Rats were paralyzed with pancuronium bromide, (0.1 mg/100 g bw/h, iv) and ventilated with 100% O₂. A Novamatrix 7000 Capnograph sensor was used to monitor end-tidal PCO₂ (PETCO₂) continuously. PETCO₂ for the entire group ranged between 35 and 38 mmHg, but within individual animals, PETCO₂ varied 2 mmHg about its mean, even including hypoxic episodes. The capnograph was calibrated weekly with a 5% CO₂ gas mixture. Arterial blood samples were analyzed for PO₂, PCO₂, and pH prior to hypoxic exposure, following the series of 10 hypoxic exposures, and at the end of the protocol; these parameters were maintained within normal limits (Radiometer ABL80).

2.2 Recorded Variables

Blood pressure, airflow, raw and integrated PNA and sSNA, as well as end-tidal PCO₂, were displayed on a chart recorder (Astro-Med Dash 8) and acquired on a computer (LabView data acquisition and analytical software written by Innovative Computer Engineering Inc.). Body temperature was maintained at approximately 37°C throughout the experiment by a servo-controlled recirculating water blanket and infrared lamps.

2.3 Experimental Protocol

The hypoxic response was elicited by challenging with 8% O₂ for 45 s. The rats were ventilated with 100% O₂ before and after the hypoxic challenge to maximize response and all hypoxic exposures were poikilocapnic. A subset of rats (n = 10) received 10 successive hypoxic challenges (45 s of 8% O₂ followed by 5 min of 100% O₂), were allowed to recover for 1h, and then were exposed to an 11th hypoxic challenge. As a control, CHH-conditioned rats (n = 3) received a single hypoxic challenge and another hypoxic challenge 2h later.

2.4 Data Analysis

Changes in the timing of the breathing pattern were analyzed by plotting sequentially the duration of expiration (TE) before, during, and after hypoxic exposure. Baseline values of TE were the average of 10 consecutive cycles before a hypoxic exposure; peak frequency (PkfR), the 3-5 successive cycles with the shortest TE; and hypoxic ventilatory depression (HVD), the 3-5 cycles with the longest TE at the end of hypoxic exposure; PHFD 3 consecutive cycles with the longest TE immediately after hypoxia; and recovery (Rcvry) 10 consecutive cycles, which occurred 60 s after hypoxia. Cycles that contained fictive swallows were excluded.

Cycle-triggered averages (CTAs) were constructed to compare the coupling patterns of PNA and sSNA. Averaging increased the signal-to-noise ratio of sSNA that was time-locked to the respiratory cycle (Dick *et al.*, 2004). For averaging, the analog signal of sSNA was rectified, integrated (CWE, Inc.; Paynter Filter, 50 ms time constant), sampled at 200 Hz and

summed (National Instruments, Analog-to-Digital board). The reference point (time zero) for cycle-triggered averages was the phase transition between inspiration and expiration. The onset of PNA was identified as the first-occurring value 10% above the baseline value for PNA and the positive slope of the integrated PNA signal. Time parameters for sampling before and after the triggering event were set so that the signal was averaged during the interval beginning 200 ms prior to the onset of Inspiration and ending 50-100 ms after the onset of the next Insp. Standard criteria used to determine the respiratory phases were established previously (Dick *et al.*, 2004). To determine the baseline pattern of sSNA and PNA synchronization, PNA and sSNA were averaged for 2-min period preceding hypoxic exposures, and baseline values were then compared to those obtained for 2-min periods at 5- and 60-min after the hypoxic exposures. The analyses included >50 consecutive cycles, and the total number of averaged cycles depended on the respiratory frequency.

The magnitudes of PNA and of sSNA and their coefficients of variation were calculated from their respective cycle-triggered averages as described in detail previously (Dick *et al.*, 2004). The “electronic noise” offsets, which were determined in the absence of nerve activity (for sSNA: electrode tips were shorted at the end of the experiment), were subtracted from the cycle-triggered averages. Measures of magnitude included: maximal and average amplitudes as well as areas under the curves were calculated for PNA and sSNA. The sSNA cycle-triggered averages were also analyzed with respect to the distribution patterns of respiratory-modulated sSNA. Inspiratory and expiratory portions of sSNA cycle-triggered averages were divided in half thereby allowing direct comparison of the magnitude of sSNA and the coefficient of variation. In addition to determining the amplitude and area of the integrated PNA, the timing variables (inspiratory and expiratory times) preceding and following intermittent hypoxia were also measured. The significance of the observed differences in sSNA, its coefficient of variation, and PNA preceding and following IH, were determined by twoway ANOVA for repeated measures, and parameters exhibiting significant differences were subjected to the Student-Newman-Keuls test to identify specific differences. To identify significant correlations among sSNA, inspiratory time, expiratory time and PNA, linear regressions of sSNA *versus* the other listed parameters during the first and second halves of each phase were performed. Values of $p < 0.05$ were taken as significant. Data are presented as means $\pm$ SD or SEM as indicated.

3. Results

3.1 Cardiorespiratory coupling before, during and after brief hypoxia in naïve rats

Respiration modulates sympathetic activity at baseline (BSLN), as well as during and after acute hypoxia. Generally, sSNA activity is greatest during the first half of expiration (BSLN, Fig.1A1 and B1; Fig. 2B). During hypoxia, the recruitment of PNA and sSNA is time-dependent. For example, the frequency (fR) of PNA bursts increases, peaking in the first 20-30 s of exposure (peak frequency, PkfR); then, decreases as hypoxic exposure continues (‘fictive’ hypoxic ventilatory decline, HVD; Fig.1A1). In contrast, $\int$ PNA increases over a longer time course and is sustained (Fig.1A1) after hypoxia; however fR decreases below baseline (Fig.1A1, double-headed arrow). During hypoxia, $\int$ sSNA varies depending on the respiratory phase; $\int$ sSNA decreases during second half of inspiration and

increases especially during the second half of expiration. Following hypoxia, sSNA becomes quiescent (PHFD; Fig. 1A1, slanted arrow) during the prolonged expiratory pause associated with the onset of post-hypoxic frequency decline (PHFD; Fig. 1A1, double-headed arrow); then as $\int$ sSNA recovers, it remains more respiratory modulated than at baseline (Fig. 1B1, compare BSLN and RCVRY panels). The changes in motor activity are consistent as described previously (Hsieh *et al.*, 2004; Hsieh *et al.*, 2008).

3.2 Cardiorespiratory coupling before, during and after brief hypoxia in CHH-conditioned rats

In comparison to *naïve* rats, respiratory modulation of sympathetic activity is attenuated at baseline (Fig. 1B1, BSLN). In the representative example, although respiratory modulation of sSNA appeared evident; it was not consistent from breath-to-breath (Fig. 1A2), in particular, during the IE transition when SNA is recruited; the standard deviation of the mean $\int$ sSNA increased (Fig. 1B2, highlighted in gray). In *naïve* rats, the standard deviation decreases in late inspiration and remains low during the post-inspiration peak (Fig. 1A1 and Fig. 2B between the arrowheads, (Dick *et al.*, 2004), At the IE transition in CHH-conditioned rats, the standard deviation became (Fig. 1B) or remained large (Fig. 2 A1 and A2). For the group of CHH-conditioned rats, sympathetic activity that was not distributed differentially within the respiratory cycle at baseline (Fig. 2C) due to a high coefficient of variation (CV) across the cycle.

The HVR is diminished and PHFD is blocked in normobaric and hypobaric hypoxic conditioned rats (Fig. 1A2, gray highlight) (Ilyinsky *et al.*, 2003; Hsieh *et al.*, 2004; Ilyinsky & Mifflin, 2005). Here, we report that respiratory modulated component of the sympathetic chemoreflex was diminished after conditioning (Figs. 1&3). Late-expiratory sSNA was not recruited until the end of the 45s exposure with HVD (Fig. 1B2 and Fig. 3A), not at peak frequency (compare Pkfr panels in Fig. 1B1 and 2).

After hypoxia, respiratory modulation of sSNA was not enhanced; and was not differentially distributed across the respiratory cycle (RCVRY, Fig. 1B2 and Fig. 3A). The unexpected observation was that sSNA persisted during PHFD (Fig. 1A2, gray highlight; see also Figs. 4E highlighted points and Fig. 5). Thus, in CHH-conditioned rats, not only was PHFD absent, but also the abrupt cessation of sSNA followed by an increase in respiratory coupling in sSNA did not occur after hypoxia.

We have emphasized that CHH conditioning diminished the *consistency* of the sympatho-respiratory coupling from breath-to-breath at baseline as well as during and after the exposure to hypoxia. To determine if averaging masked a variable response, we analyzed the maximal evoked $\int$ sSNA. Surprisingly, in both *naïve* and CHH-conditioned rats, $\int$ sSNA_{max} increased similarly during hypoxia (Figs. 4D and 4E). Whether we compared absolute values (Fig. 4A) or normalized them to baseline (Fig. 4E) significant increases in maximal evoked activity indicated that a chemoreflex was present. After hypoxia, the maximal amplitude of $\int$ sSNA was greater in CHH than *naïve* rats (Fig. 4E).

3.3 Plasticity evoked by acute intermittent hypoxia (AIH) attenuated in CHH-conditioned rats

Generally, AIH elicits LTF in PNA and sSNA (Ling *et al.*, 2001; Dick *et al.*, 2007). In fact, sSNA is more robust than that of PNA (Xing & Pilowsky, 2010; Xing *et al.*, 2013). However, CHH-conditioned rats fail to exhibit IH-induced LTF in the respiratory pattern (Hsieh *et al.*, 2008). Here, we test whether IH could restore the HVR and PHFD (Fig. 6). Consistent with the attenuated respiratory LTF, the hypoxic response of sSNA was comparable before, during, and after the first, tenth and eleventh hypoxic responses (Fig. 6). Although the amplitude of PNA increases after the first and tenth hypoxic challenges, this increased activity neither grows nor persists in the following 60 min. Thus, $\int$ PNA amplitude during the eleventh is similar to that during the first hypoxic challenge. Similarly, respiratory modulation of SNA did not increase either immediately after a single exposure (Fig. 6 compare BSLN to RCVRY for HR1, 10 or 11) or progressively after multiple exposures (Fig 6; compare BSLN for HR1 to BSLN for HR11). Furthermore, sympathetic and respiratory motor activities are not correlated (not shown). Take together these results indicate that after conditioning to CHH, acute IH is unable to restore the HVR and sympathetic chemoreflex observed in *naïve* rats.

Discussion

CHH conditioning attenuates the relationship between sSNA and respiratory pattern at baseline, during and following hypoxia. These findings complement our previous report that the HVR was attenuated and LTF was not evoked after CHH conditioning (Hsieh *et al.*, 2004; Hsieh *et al.*, 2008). Here, we associate the attenuated HVR with an absence of respiratory modulation at baseline due to variability sympatho-respiratory coupling, a delay of phase-specific recruitment during hypoxia, persistence of sSNA without enhanced respiratory modulation immediately after hypoxia. We also report that repeated hypoxic exposures did not re-establish the HVR, the sympathetic chemoreflex or the respiratory-modulation of sSNA. These findings are consistent with the interpretation that the strength of sympatho-respiratory coupling is weakened by CHH-conditioning and that activation of sSNA, especially the short-term, activity dependent plasticity of sSNA is mediated through its interaction with the respiratory control system.

We associate the absence of respiratory-modulation of sSNA at baseline with suppression of sympatho-respiratory plasticity. The effects of CHH on plasticity did not result from blocking the sympathetic chemoreflex *per se*, as maximal sSNA was similar in *naïve* and CHH-conditioned rats. The comparable maximal sSNA is consistent with the tonic component of sympathetic chemoreflex being unaffected by CHH conditioning. If the weakened sympatho-respiratory coupling unmasks the tonic component of the sympathetic chemoreflex and allows persistent activity after hypoxia, then this component in and of itself does not appear to generate sympathetic LTF in the absence of respiratory LTF. This hypothesis could be tested directly by performing experiments as described by Koshiya and Guyenet in CHH-conditioned rats (Koshiya & Guyenet, 1996). They injected muscimol, a GABAA receptor agonist, into the ventrolateral medulla bilaterally. This silenced PNA at rest and during chemoreceptor stimulation but did not change the mean increase in sSNA

produced by chemoreceptor stimulation (Koshiya & Guyenet, 1996). These experiments would distinguish whether CHH-conditioning affects only the respiratory-modulated component of the sympathetic chemoreflex.

The attenuation of hypoxia-evoked plasticity denotes a form of metaplasticity, the concept that neural control networks can alter their expression of plasticity (Abraham & Bear, 1996). Previous reports have identified metaplasticity in the respiratory control system (Ilyinsky *et al.*, 2003; Mitchell & Johnson, 2003; Ilyinsky & Mifflin, 2005). Respiratory metaplasticity has been observed previously, including an enhanced phrenic LTF following CIH conditioning (Mitchell & Johnson, 2003), and a decreased PHFD following chronic normobaric hypoxia (10% O₂ exposure for 6-14 days (Ilyinsky *et al.*, 2003; Ilyinsky & Mifflin, 2005). We speculate that the respiratory-modulated component of the sympathetic chemoreflex confers the properties of plasticity and metaplasticity on sympathetic activity. This speculation is based on both the expression of 'short-term potentiation' in sSNA immediately after hypoxia and the absence of LTF in sSNA after repeated hypoxia.

Metaplasticity in cardiorespiratory control may play a role in adaptation to high altitude. In humans and animals living at high altitude, the adaptation of the neural control of the cardio-respiratory system varies across populations and species. The HVR can be augmented or blunted (Severinghaus *et al.*, 1966; Sorensen & Severinghaus, 1968). Native highlanders, Himalayans, Andeans, and Ethiopians, have developed different repertoires of adaptive sympatho-respiratory neural control (Beall *et al.*, 1997; Beall *et al.*, 2002). Differential acclimatization is evident in rodent strains and even in the same strain but from different vendors. In fact, the hypoxic responses of rats appear to be particularly variable. In Sprague-Dawley rats from Zivic Miller, conditioning with CHH suppressed the burst frequency response to hypoxia (Hsieh *et al.*, 2004; Hsieh *et al.*, 2008). In contrast, Sprague-Dawley rats from Charles River Laboratories and Harlan exhibited increased, rather than decreased ventilatory responsiveness after chronic hypoxic conditioning (Aaron & Powell, 1993; Dwinell & Powell, 1999). Differences in experimental conditions (e.g. severity, duration, and type) between the present and previous studies (Dwinell & Powell, 1999; Reeves *et al.*, 2003) might also contribute to these discrepancies in hypoxic response. Other rats such as Wistars (Tucks) do exhibit a blunted hypoxic response (Wach *et al.*, 1989). Nevertheless, the present findings in rats are relevant, since other species, including humans, exhibit attenuated hypoxic responses following CHH (White *et al.*, 1987; Weil, 1994; Zabka *et al.*, 2001; Hupperets *et al.*, 2004).

The mechanisms involved in our observed sympatho-respiratory metaplasticity are unknown, but probably involve modifications of central neurotransmitters. In previous reports, we correlated the effects of CHH conditioning with increased expression of GABA_A receptor subunits $\alpha 4$, $\alpha 6$ and δ (Hsieh *et al.*, 2004; Hsieh *et al.*, 2008). The changes in subunit expression were specific to the pons (Hsieh *et al.*, 2004; Hsieh *et al.*, 2008), and primarily in the sympatho-respiratory control nuclei that may mediate the response to hypoxia, PHFD and LTF (Hsieh *et al.*, 2008). These subunits are thought to be involved in volumetric, non-synaptic GABA-mediated neurotransmission, and regulating tonic conductance (Mody, 2001). Gozal and coworkers observed altered expression of NMDA

receptors in the dorsocaudal brainstem after conditioning with various types of hypoxic challenges (Reeves *et al.*, 2003).

Conclusions

Obstructive sleep apnea (OSA) involves intermittent apneic episodes that can lead to hypertension, may be a consequence of the metaplasticity of the sympatho-respiratory motor system. CIH-conditioned rats, a model that mimics the hypertension associated with OSA, express sustained and elevated sympatho-respiratory activity and increased blood pressure (Fletcher, 2000; Prabhakar *et al.*, 2005). Such short- and long-term plasticities expressed in response to hypoxia may be interpreted as the adaptations of the cardiorespiratory control system to perturbation. In the present studies, conditioning to CHH attenuated PHFD and LTF in both sympathetic and respiratory systems. The blunted activity-dependent plasticity of our CHH-conditioned rats was not reversed by IH, suggesting that CHH conditioning exerts a “protective” effect against intermittent hypoxia. This further suggests that in human populations native to high altitudes and with blunted hypoxic responses, acclimatization to lower ambient PO₂ protects against systemic hypertension evoked by OSA. Therefore, elucidating the mechanisms involved in mediating the altered sensitivity to hypoxia caused by conditioning to chronic and repetitive hypoxia may provide insight into the pathophysiology of hypertension.

Acknowledgments

The authors gratefully thank Dr. Jeffery Tatro and Dr. Stephen Lewis for his assistance in critically reading the manuscript.

Grants: We gratefully acknowledge that this work was supported by T32 HL007913, NS069220, HL-080318, and VA I01BX000873.

References

- Aaron EA, Powell FL. Effect of chronic hypoxia on hypoxic ventilatory response in awake rats. *J Appl Physiol.* 1993; 74:1635–1640. [PubMed: 8514677]
- Abdala AP, Rybak IA, Smith JC, Zoccal DB, Machado BH, St-John WM, Paton JF. Multiple pontomedullary mechanisms of respiratory rhythmogenesis. *Respir Physiol Neurobiol.* 2009; 168:19–25. [PubMed: 19540366]
- Abraham WC, Bear MF. Metaplasticity: the plasticity of synaptic plasticity. *Trends Neurosci.* 1996; 19:126–130. [PubMed: 8658594]
- Aubert AE, Seps B, Beckers F. Heart rate variability in athletes. *Sports Med.* 2003; 33:889–919. [PubMed: 12974657]
- Baker TL, Fuller DD, Zabka AG, Mitchell GS. Respiratory plasticity: differential actions of continuous and episodic hypoxia and hypercapnia. *Respir Physiol.* 2001; 129:25–35. [PubMed: 11738644]
- Baker TL, Mitchell GS. Episodic but not continuous hypoxia elicits long-term facilitation of phrenic motor output in rats. *J Physiol.* 2000; 529(Pt 1):215–219. [PubMed: 11080263]
- Beall CM, Decker MJ, Brittenham GM, Kushner I, Gebremedhin A, Strohl KP. An Ethiopian pattern of human adaptation to high-altitude hypoxia. *Proc Natl Acad Sci U S A.* 2002; 99:17215–17218. [PubMed: 12471159]
- Beall CM, Strohl KP, Blangero J, Williams-Blangero S, Almasy LA, Decker MJ, Worthman CM, Goldstein MC, Vargas E, Villena M, Soria R, Alarcon AM, Gonzales C. Ventilation and hypoxic ventilatory response of Tibetan and Aymara high altitude natives. *Am J Phys Anthropol.* 1997; 104:427–447. [PubMed: 9453694]

- Coles SK, Dick TE. Neurones in the ventrolateral pons are required for post-hypoxic frequency decline in rats. *J Physiol.* 1996; 497(Pt 1):79–94. [PubMed: 8951713]
- Dick TE, Hsieh YH, Morrison S, Coles SK, Prabhakar N. Entrainment pattern between sympathetic and phrenic nerve activities in the Sprague-Dawley rat: hypoxia-evoked sympathetic activity during expiration. *Am J Physiol Regul Integr Comp Physiol.* 2004; 286:R1121–1128. [PubMed: 15001434]
- Dick TE, Hsieh YH, Wang N, Prabhakar N. Acute intermittent hypoxia increases both phrenic and sympathetic nerve activities in the rat. *Exp Physiol.* 2007; 92:87–97. [PubMed: 17138622]
- Dick TE, Molkov YI, Nieman G, Hsieh YH, Jacono FJ, Doyle J, Scheff JD, Calvano SE, Androulakis IP, An G, Vodovotz Y. Linking Inflammation, Cardiorespiratory Variability, and Neural Control in Acute Inflammation via Computational Modeling. *Front Physiol.* 2012; 3:222. [PubMed: 22783197]
- Dwinell MR, Powell FL. Chronic hypoxia enhances the phrenic nerve response to arterial chemoreceptor stimulation in anesthetized rats. *J Appl Physiol.* 1999; 87:817–823. [PubMed: 10444644]
- Fletcher EC. Effect of episodic hypoxia on sympathetic activity and blood pressure. *Respir Physiol.* 2000; 119:189–197. [PubMed: 10722862]
- Fuller DD, Bach KB, Baker TL, Kinkead R, Mitchell GS. Long term facilitation of phrenic motor output. *Respir Physiol.* 2000; 121:135–146. [PubMed: 10963770]
- Fuller DD, Golder FJ, Olson EB Jr, Mitchell GS. Recovery of phrenic activity and ventilation after cervical spinal hemisection in rats. *J Appl Physiol.* 2006; 100:800–806. [PubMed: 16269524]
- Fuller DD, Johnson SM, Olson EB Jr, Mitchell GS. Synaptic pathways to phrenic motoneurons are enhanced by chronic intermittent hypoxia after cervical spinal cord injury. *J Neurosci.* 2003; 23:2993–3000. [PubMed: 12684486]
- Garcia AJ 3rd, Koschnitzky JE, Dashevskiy T, Ramirez JM. Cardiorespiratory coupling in health and disease. *Auton Neurosci.* 2013; 175:26–37. [PubMed: 23497744]
- Golder FJ, Mitchell GS. Spinal synaptic enhancement with acute intermittent hypoxia improves respiratory function after chronic cervical spinal cord injury. *J Neurosci.* 2005; 25:2925–2932. [PubMed: 15772352]
- Guyenet PG, Koshiya N. Working model of the sympathetic chemoreflex in rats. *Clin Exp Hypertens.* 1995; 17:167–179. [PubMed: 7735267]
- Hsieh YH, Dick TE, Siegel RE. Adaptation to hypobaric hypoxia involves GABA A receptors in the pons. *Am J Physiol Regul Integr Comp Physiol.* 2008; 294:R549–557. [PubMed: 18056985]
- Hsieh YH, Siegel RE, Dick TE. Pontine GABAergic pathways: role and plasticity in the hypoxic ventilatory response. *Respir Physiol Neurobiol.* 2004; 143:141–153. [PubMed: 15519551]
- Hupperets MD, Hopkins SR, Pronk MG, Tiemessen IJ, Garcia N, Wagner PD, Powell FL. Increased hypoxic ventilatory response during 8 weeks at 3800 m altitude. *Respir Physiol Neurobiol.* 2004; 142:145–152. [PubMed: 15450476]
- Ilyinsky O, Mifflin S. Chronic hypoxia abolishes expiratory prolongation following carotid sinus nerve stimulation in the anesthetized rat. *Respir Physiol Neurobiol.* 2005; 146:269–277. [PubMed: 15766915]
- Ilyinsky O, Tolstykh G, Mifflin S. Chronic hypoxia abolishes posthypoxia frequency decline in the anesthetized rat. *Am J Physiol Regul Integr Comp Physiol.* 2003; 285:R1322–1330. [PubMed: 14615401]
- Koshiya N, Guyenet PG. Tonic sympathetic chemoreflex after blockade of respiratory rhythmogenesis in the rat. *J Physiol.* 1996; 491(Pt 3):859–869. [PubMed: 8815217]
- Ling L, Fuller DD, Bach KB, Kinkead R, Olson EB Jr, Mitchell GS. Chronic intermittent hypoxia elicits serotonin-dependent plasticity in the central neural control of breathing. *J Neurosci.* 2001; 21:5381–5388. [PubMed: 11438615]
- Millhorn DE, Eldridge FL, Waldrop TG. Prolonged stimulation of respiration by a new central neural mechanism. *Respir Physiol.* 1980a; 41:87–103. [PubMed: 6771859]
- Millhorn DE, Eldridge FL, Waldrop TG. Prolonged stimulation of respiration by endogenous central serotonin. *Respir Physiol.* 1980b; 42:171–188. [PubMed: 6452673]

- Mitchell GS, Johnson SM. Neuroplasticity in respiratory motor control. *J Appl Physiol.* 2003; 94:358–374. [PubMed: 12486024]
- Mody I. Distinguishing between GABA(A) receptors responsible for tonic and phasic conductances. *Neurochem Res.* 2001; 26:907–913. [PubMed: 11699942]
- Moraes DJ, Bonagamba LG, Costa KM, Costa-Silva JH, Zoccal DB, Machado BH. Short-term sustained hypoxia induces changes in the coupling of sympathetic and respiratory activities in rats. *J Physiol.* 2014
- Poon CS, Siniaia MS. Plasticity of cardiorespiratory neural processing: classification and computational functions. *Respir Physiol.* 2000; 122:83–109. [PubMed: 10967337]
- Powell FL, Milsom WK, Mitchell GS. Time domains of the hypoxic ventilatory response. *Respir Physiol.* 1998; 112:123–134. [PubMed: 9716296]
- Prabhakar NR, Peng YJ, Jacono FJ, Kumar GK, Dick TE. Cardiovascular alterations by chronic intermittent hypoxia: importance of carotid body chemoreflexes. *Clin Exp Pharmacol Physiol.* 2005; 32:447–449. [PubMed: 15854156]
- Reeves SR, Gozal E, Guo SZ, Sachleben LR Jr, Brittian KR, Lipton AJ, Gozal D. Effect of long-term intermittent and sustained hypoxia on hypoxic ventilatory and metabolic responses in the adult rat. *J Appl Physiol.* 2003; 95:1767–1774. [PubMed: 14555663]
- Severinghaus JW, Bainton CR, Carcelen A. Respiratory insensitivity to hypoxia in chronically hypoxic man. *Respir Physiol.* 1966; 1:308–334. [PubMed: 5968347]
- Song G, Poon CS. Functional and structural models of pontine modulation of mechanoreceptor and chemoreceptor reflexes. *Respir Physiol Neurobiol.* 2004; 143:281–292. [PubMed: 15519561]
- Sorensen SC, Severinghaus JW. Irreversible respiratory insensitivity to acute hypoxia in man born at high altitude. *J Appl Physiol.* 1968; 25:217–220. [PubMed: 5669868]
- Wach RA, Bee D, Barer GR. Dopamine and ventilatory effects of hypoxia and almitrine in chronically hypoxic rats. *J Appl Physiol.* 1989; 67:186–192. [PubMed: 2569453]
- Weil JV. Ventilatory responses to CO₂ and hypoxia after sustained hypoxia in awake cats. *J Appl Physiol.* 1994; 76:2251–2252. [PubMed: 7928843]
- White DP, Gleeson K, Pickett CK, Rannels AM, Cymerman A, Weil JV. Altitude acclimatization: influence on periodic breathing and chemoresponsiveness during sleep. *J Appl Physiol.* 1987; 63:401–412. [PubMed: 3114224]
- Xing T, Fong AY, Bautista TG, Pilowsky PM. Acute intermittent hypoxia induced neural plasticity in respiratory motor control. *Clin Exp Pharmacol Physiol.* 2013; 40:602–609. [PubMed: 23781949]
- Xing T, Pilowsky PM. Acute intermittent hypoxia in rat in vivo elicits a robust increase in tonic sympathetic nerve activity that is independent of respiratory drive. *J Physiol.* 2010; 588:3075–3088. [PubMed: 20566662]
- Zabka AG, Behan M, Mitchell GS. Long term facilitation of respiratory motor output decreases with age in male rats. *J Physiol.* 2001; 531:509–514. [PubMed: 11230522]
- Zoccal DB, Paton JF, Machado BH. Do changes in the coupling between respiratory and sympathetic activities contribute to neurogenic hypertension? *Clin Exp Pharmacol Physiol.* 2009; 36:1188–1196. [PubMed: 19413588]

Highlights

- Chronic hypobaric hypoxia attenuates respiratory modulation of sympathetic activity.
- Respiratory modulation of sympathetic activity is inconsistent from breath-to-breath.
- Respiratory and sympathetic motor activities do not express their dynamics during and after hypoxia.
- Repetitive acute hypoxic exposures does not restore the hypoxic response, evoke long-term facilitation and enhance the respiratory-modulation of sympathetic activity.

Figure 1.

Conditioning to chronic hypobaric hypoxia (CHH) alters the sympatho-respiratory response to acute hypoxia. **A.** Representative tracings from a *naïve* (1) and a CHH-conditioned (2) rat exhibiting their sympatho-respiratory motor patterns during and after hypoxia. **A.1.** and **A.2.** Tracings from top to bottom: rectified and integrated splanchnic sympathetic (sSNA) and phrenic nerve activity (PNA). **A.1.** In a *naïve* rat, the response to hypoxia consists of an initial increase in peak frequency (P_kF) followed by a decrease in frequency (hypoxic ventilatory decline, HVD). Immediately after hypoxia, frequency decreased below baseline (post-hypoxic frequency decline (PHFD, two-headed red arrow) and then recovered (RCVRY, not shown) to baseline (BSLN) within 5 min. Splanchnic sympathetic nerve activity is recruited throughout hypoxia but abruptly becomes quiescent during PHFD (one-headed gray arrow). **A.2.** In a CHH-conditioned rat, the sympatho-respiratory coupling sympatho-respiratory hypoxic response is attenuated. Although the response is present, PHFD is markedly attenuated and sympathetic activity persists immediately after hypoxia. **B.** Cycle-triggered averages corresponding to sympatho-respiratory components of the hypoxic response using the abrupt decrease in $\int$ PNA as the reference event. **B.1.** In *naïve* rats, sSNA was recruited throughout the hypoxic response. After hypoxia, sSNA was reduced during PHFD and both magnitude and respiratory-modulation of sSNA increased with recovery (compare activity between arrow heads). **B.2.** In CHH-conditioned rats, although sSNA appeared to burst during post-hypoxic period before hypoxia, this modulation was weak as shown by the flat negative standard deviation (highlighted in gray). During hypoxia, sSNA was recruited. Further sSNA remained active at the transition from hypoxia to hyperoxia (during PHFD), and respiratory modulation of sSNA did not increase.

Figure 2.

Respiratory modulation of sSNA in CHH-conditioned rats is reduced at baseline. **A. and B.** Cycle-triggered averages, and **C.** mean ($\pm$ SD) of $\int$ sSNA in the first and second halves of inspiration (I) and expiration (E). **Fig 2. A.1. and A.2.** Cycle-triggered averages of $\int$ sSNA at baseline from CHH-conditioned rats. The magnitude of the standard deviation does not change with the respiratory cycle, in particular even at the 2nd half of inspiration or the first half of expiration (double headed red arrows). **Fig. 2B.** A cycle-triggered average of $\int$ sSNA at baseline from a naïve rat. Standard deviation decreases in the 2nd half of inspiration or the first half of expiration (red arrows). **Fig. C.** Bar graphs of mean activity and the coefficient of variability (CV) $\pm$ SEM in both halves of inspiration and expiration in CHH-conditioned rats. No statistical differences across the phase in either mean activity or the CV.

Figure 3.

Respiratory modulation of hypoxia-induced sSNA in CHH-conditioned rats is reduced: Mean amplitude (A) and Coefficient of Variation (CV) (B) of sSNA before, during and after the response to hypoxia. Sympathetic activity throughout the respiratory phase was divided amongst Inspiratory (I) and Expiratory (E) phase. Within I and E, amplitude of sympathetic activity was divided into 2 phases (sI-1, sI-2, sE-1, & sE-2). **A.** At Pkfr, mean sSNA amplitude was significantly lower during the 2nd half of I than during the other periods (asterisk, arrow). During the HVD, sSNA during I was significantly lower than during E. Also activity during the 2nd half of E was significantly greater than that during the corresponding periods of BSLN, PHFD, and RCVRY (Asterisk and lines below x axis). During PHFD, sSNA during the 2nd half of I was lower than during the 1st half of E. By Rcvry, sSNA magnitude and distribution were not statistically different within the respiratory cycle and were similar to that at BSLN (mean $\pm$ SD). $\ast=p<0.05$. **B.** The CV,

which is indicative of the consistency of the respiratory modulation of sSNA, was not differentially distributed and did not change significantly during or after the hypoxic response.

Figure 4.

Sympatho-respiratory variables and responses to acute hypoxia are attenuated by CHH-conditioning. **A.** For CHH-conditioned animals, inspiratory time (TI) (◆), expiratory time (TE) (●), maximal values of integrated sSNA (×) and PNA (x025B2)), are plotted at BSLN, PkF and HVD during hypoxia, and post-hypoxic frequency decline (PHFD) and a minute later (recovery, RCVRY). TE is significantly shorter during peak frequency (PkF) and lengthened during PHFD than at the other points (Solid circles surround values that are significantly different from the others). The PNA_{max} and $sSNA_{max}$ (right axis) increase during hypoxia. Furthermore, during hypoxic ventilatory decline (HVD), $sSNA_{max}$ is significantly greater than that during baseline (BSLN) and RCVRY (Dashed gray circles surround values that are significantly different only from each other). Values are mean $\pm$ SEM. (*= $p < 0.05$). **B- E.** Values normalized to baseline are compared between *naïve* (◆) and CHH-conditioned (◇) rats. Conditioned rats exhibit attenuated TE (panel B) and increased $sSNA_{max}$ during PHFD (panel E) relative to *naïve* rats (Dashed gray circles surround values that are significantly different only from each other). Normalized TI values (Panel C) and maximal PNA values (Panel D) do not have significant differences between *naïve* and CHH rats

Figure 5.

Example of paired tracings of $\int$ sSNA (top) and $\int$ PNA (bottom) in individual representative CHH-conditioned (**Panels A.1. and A.2.**) and naïve (**Panel B**) rats during the transition from hypoxia to hyperoxia (inhaled gases switched at dashed line). **Panel A.** In CHH-conditioned rats sSNA persisted during the transition from hypoxia to hyperoxia and PHFD was blocked. **Panel B.** In naïve rats, sSNA was quiescent during the transition.

Figure 6.

Sympatho-respiratory responses of a representative *naïve* and CHH-conditioned animal to acute intermittent hypoxia. Hypoxic responses of $\int$ sSNA and $\int$ PNA to the initial exposure (HR 1), followed by nine challenges (HR 10), and after 1 h (HR 11). HR 1, 10, and 11 are comparable, indicating that long-term facilitation was not evoked by IH in CHH-conditioned rats. In particular, a blunted sympatho-respiratory response to acute hypoxia was preserved throughout the hypoxic challenges.