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Ventilatory and chemoreceptor responses to hypercapnia in neonatal rats chronically exposed to moderate hyperoxia

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ABSTRACT

Rats reared in hyperoxia hypoventilate in normoxia and exhibit progressive blunting of the hypoxic ventilatory response, changes which are at least partially attributed to abnormal carotid body development. Since the carotid body also responds to changes in arterial CO_2/pH, we tested the hypothesis that developmental hyperoxia would attenuate the hypercapnic ventilatory response (HCVR) of neonatal rats by blunting peripheral and/or central chemoreceptor responses to hypercapnic challenges. Rats were reared in 21% O_2 (Control) or 60% O_2 (Hyperoxia) until studied at 4, 6–7, or 13–14 days of age. Hyperoxia rats had significantly reduced single-unit carotid chemoafferent responses to 15% CO_2 at all ages; CO_2 sensitivity recovered within 7 days after return to room air. Hypercapnic responses of CO_2-sensitive neurons of the caudal nucleus tractus solitarius (cNTS) were unaffected by chronic hyperoxia, but there was evidence for a small decrease in neuronal excitability. There was also evidence for augmented excitatory synaptic input to cNTS neurons within brainstem slices. Steady-state ventilatory responses to 4% and 8% CO_2 were unaffected by developmental hyperoxia in all three age groups, but ventilation increased more slowly during the normocapnia-to-hypercapnia transition in 4-day-old Hyperoxia rats. We conclude that developmental hyperoxia impairs carotid body chemosensitivity to hypercapnia, and this may compromise protective ventilatory reflexes during dynamic respiratory challenges in newborn rats. Impaired carotid body function has less of an impact on the HCVR in older rats, potentially reflecting compensatory plasticity within the CNS.

1. Introduction

Development of the respiratory control system is influenced by the oxygen levels experienced during early life (Carroll, 2003; Bavis, 2005). Neonatal rats exposed to chronic hyperoxia hypoventilate when returned to room air (Bavis et al., 2010, 2014b) and exhibit a progressive blunting of their hypoxic ventilatory response (HVR) over the course of the hyperoxic exposure (Bavis et al., 2010). The blunted HVR is an example of developmental plasticity, defined as a form of phenotypic plasticity that is unique to developing organisms (Bavis and Mitchell, 2008). Indeed, long-lasting blunting of the HVR only occurs when rats experience hyperoxia during the first two postnatal weeks (Ling et al., 1996; Bavis et al., 2002) and persists for months after return to room air (Ling et al., 1996; Fuller et al., 2002). Chronic hyperoxia is thought to blunt the HVR by causing abnormal development of the carotid body, the principal site for arterial O_2 chemoreception (Bavis et al., 2013). Hyperoxia-treated individuals have fewer O_2-sensitive glomus (type I) cells in their carotid bodies and fewer chemoafferent axons in the associated carotid sinus nerve (CSN) (Erickson et al., 1998; Dmitrieff et al., 2012; Chavez-Valdez et al., 2012); these morphological changes lead to lifelong reductions in whole-nerve CSN responses to hypoxia (Fuller et al., 2002; Bisgard et al., 2003). The surviving glomus cells are also less responsive to hypoxia when tested immediately after the hyperoxic exposure (Donnelly et al., 2005, 2009; Bavis et al., 2011; Kim et al., 2013), but their O_2 sensitivity recovers shortly after return to normoxia (Bavis et al., 2011).

In contrast to lifelong impairment of the HVR, previous studies have not detected plasticity in the hypercapnic ventilatory response (HCVR) in mammals after developmental hyperoxia.

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Indeed, the HCVR was normal in adult rats (Ling et al., 1996) and adult mice (Dauger et al., 2003) that had been exposed to 60–65% O_2 for the first month of life. However, the HCVR has never been assessed for hyperoxia-treated rats *as neonates*. This is potentially important since some respiratory effects of developmental hyperoxia may only be apparent during the neonatal period. For example, neonatal rats hypoventilate and exhibit reduced carotid body glomus cell O_2 sensitivity immediately after chronic hyperoxia, but neither of these effects persist after a few days in room air (Bavis et al., 2011, 2014b). Moreover, chickens incubated in hyperoxia exhibit a modest reduction in the HCVR when tested shortly after hatching (Mortola, 2011).

Developmental hyperoxia has the potential to alter both peripheral and central components of the respiratory control system responsible for the HCVR. Carotid body glomus cells depolarize in response to increasing CO_2 and decreasing pH in the arterial blood, ultimately resulting in neurotransmitter secretion and the activation of afferent neurons that conduct action potentials to the nucelus tractus solitarius (NTS) in the brainstem (Lahiri and Forster, 2003; Kumar and Prabhakar, 2012). Across a range of species and experimental approaches, carotid body responses have been estimated to explain up to one-third of the increase in ventilation during hypercapnic challenges, particularly during mild hypercapnia (Forster and Smith, 2010; Smith et al., 2010). Carotid bodies also drive the initial ventilatory increase observed in neonatal rats immediately after a change in inspired CO_2 (Cummings and Frappell, 2009). Therefore, hyperoxia-induced carotid body hypoplasia and loss of carotid chemoafferent neurons could directly impact the HCVR, as could any changes to glomus cell CO_2/pH sensitivity. Moreover, there is evidence that carotid chemoreceptor activity modulates the CO_2 sensitivity of central chemoreceptors (i.e., central and peripheral chemoreceptors are "interdependent"; cf. Forster and Smith, 2010; Smith et al., 2010). This model is supported by neuroanatomical and neurophysiological studies showing that (1) neurons in the NTS project to the retrotrapezoid nucleus (RTN), one of the putative sites for central CO_2 sensitivity (Takakura et al., 2006; Guyenet et al., 2009) and (2) many NTS neurons are themselves CO_2-sensitive chemoreceptors (Conrad et al., 2009). However, the generality of this interaction across species and physiological states and its ultimate effect on respiratory control (i.e., additive, hypoadditive, or hyperadditive) remains a matter of debate (e.g., Duffin and Mateika, 2013; Teppema and Smith, 2013; Wilson and Day, 2013; Cummings, 2014).

Central CO_2 chemoreceptors are located throughout the brainstem and include neurons in the NTS, dorsal motor neucleus of vagus (DMV), RTN, medullary raphé, rostroventrolateral medulla, pre-Bötzinger complex, and locus coeruleus (LC) (Dean and Putnam, 2010; Nattie and Li, 2012). The integrated activity from these neuronal populations is the primary determinant of the whole-animal HCVR. Importantly, hyperoxia may alter the behavior of at least some of these cell populations. Acute exposure to normobaric or hyperbaric hyperoxia (i.e., minutes to hours) stimulates CO_2-sensitive and CO_2-insensitive neurons of the NTS and DMV and repeated exposure to hyperoxia may diminish CO_2 sensitivity (Dean and Putnam, 2010; Matott et al., 2014). Therefore, the impact of developmental hyperoxia on CO_2-sensitive neurons in the NTS warrants further investigation.

In the present study, we tested the hypothesis that developmental hyperoxia would attenuate the HCVR of neonatal rats by blunting peripheral and/or central chemoreceptor responses to hypercapnic challenges. Specifically, neonatal rats were exposed to 60% O_2 from birth and the HCVR, peripheral (carotid body) CO_2 chemosensitivity, and central (NTS) CO_2 chemosensitivity were examined at several ages during the first two weeks of life (P4–P14). This age range was selected to encompass much of the postnatal maturation of the HCVR (Stunden et al., 2001; Putnam et al., 2005) and the critical window for hyperoxia-induced carotid body plasticity (Bavis et al., 2002; Bavis et al., 2013) in rats.

2. Methods

2.1. Experimental animals

Experiments were conducted on Sprague-Dawley rat pups of both sexes. Timed-pregnant rats were obtained from a commercial supplier (Charles River Laboratories; Portage, MI, USA) and placed into environmental chambers on the day before they were expected to give birth. Chambers were flushed with room air and oxygen at sufficient flow rates to maintain 60% O_2. The resulting pups ("Hyperoxia") were raised in 60% O_2 with their mothers until studied (i.e., 4–14 days of age (P4–P14), where P0 is the day of birth). Additional litters were reared in identical chambers flushed with 21% O_2 to serve as age-matched control groups ("Control"). One Hyperoxia litter was removed from the chamber when pups reached P7 and permitted to recover in room air prior to study at P13–15 ("Recovery"). All rats were maintained on a 12:12 light cycle throughout the study and provided food and water ad libitum.

All experimental procedures were approved by the Institutional Animal Care and Use Committees at Bates College (carotid chemoafferent nerve recordings and ventilation measurements) and Wright State University (NTS brainstem slice recordings).

2.2. Single-unit carotid chemoafferent nerve recordings

Carotid chemoafferent nerve recordings were made at P4, P7, and P14 (or P13–15 for the Recovery group) using the same methods used in our previous studies (e.g., Bavis et al., 2011). Recordings were made from pups derived from 5 to 8 different litters per treatment group at each age, except for the Recovery group which consisted of pups from one litter; the number of recordings made for each group is reported in Fig. 2.

Rat pups were deeply anesthetized by exposure to 100% CO_2 and then decapitated. The left and right carotid bifurcations (containing the carotid bodies) and nodose-petrosal ganglion complexes were isolated *en bloc* and placed into an ice-cold, oxygenated (95% O_2, 5% CO_2) saline solution containing (in mM): 125 NaCl, 5 KCl, 2 $CaCl_2$, 1 Na_2HPO_4, 1 $MgSO_4$, 26 $NaHCO_3$, and 5 dextrose. The preparation was cleaned initially using a solution of 0.15–0.25% collagenase (collagenase P; Roche Diagnostics) and 0.01% protease (type XIV; Sigma-Aldrich) in saline (30 min at 37 °C), and then cleaned manually (in ice-cold saline) until only the carotid body, carotid sinus nerve (CSN), glossopharyngeal nerve, and petrosal ganglion remained. The preparation was transferred to a perfusion chamber (RC-22C; 140 μl; Warner Instruments) on the stage of an inverted microscope (Eclipse TE-300; Nikon). The chamber was perfused with saline solution equilibrated with 21% O_2 and 5% CO_2 (balance N_2) and delivered at a rate of ~3 ml min^{-1} through stainless steel tubing. The solution was passed through an in-line heater (SH-27B; Warner Instruments) so that bath temperature was 37° C.

Single-unit activity was recorded from the soma of carotid chemoafferent neurons using a suction electrode advanced into the petrosal ganglion. The pipette potential was amplified 2000–5000× with an extracellular amplifier (EX-1; Dagan Instruments), passband-filtered (0.1–2 kHz), and digitized (10 kHz sample rate; Powerlab 8/30 and Chart 5.2 software; ADInstruments). For identification of individual chemoafferent cells under baseline conditions (21% O_2, 5% CO_2, balance N_2), the carotid body was stimulated (~200 μA × 0.05 ms pulse duration) at 0.5–1 Hz (Isostim A320; World Precision Instruments) using a glass electrode (filled with 1 M NaCl). If a stimulus elicited a single orthodromic action potential, the stimulator was turned off and baseline nerve