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Postnatal development of eupneic ventilation and metabolism in rats chronically exposed to moderate hyperoxia

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ABSTRACT

Newborn rats chronically exposed to moderate hyperoxia (60% O_2) exhibit abnormal respiratory control, including decreased eupneic ventilation. To further characterize this plasticity and explore its proximate mechanisms, rats were exposed to either 21% O_2 (Control) or 60% O_2 (Hyperoxia) from birth until studied at 3–14 days of age (P3–P14). Normoxic ventilation was reduced in Hyperoxia rats when studied at P3, P4, and P6–7 and this was reflected in diminished arterial O_2 saturations; eupneic ventilation spontaneously recovered by P13–14 despite continuous hyperoxia, or within 24 h when Hyperoxia rats were returned to room air. Normoxic metabolism was also reduced in Hyperoxia rats but could be increased by raising inspired O_2 levels (to 60% O_2) or by uncoupling oxidative phosphorylation within the mitochondrion (2,4-dinitrophenol). In contrast, moderate increases in inspired O_2 had no effect on sustained ventilation which indicates that hypoventilation can be dissociated from hypometabolism. The ventilatory response to abrupt O_2 inhalation was diminished in Hyperoxia rats at P4 and P6–7, consistent with smaller contributions of peripheral chemoreceptors to eupneic ventilation at these ages. Finally, the spontaneous respiratory rhythm generated in isolated brainstem-spinal cord preparations was significantly slower and more variable in P3–4 Hyperoxia rats than in age-matched Controls. We conclude that developmental hyperoxia impairs both peripheral and central components of eupneic ventilatory drive. Although developmental hyperoxia diminishes metabolism as well, this appears to be a regulated hypometabolism and contributes little to the observed changes in ventilation.

1. Introduction

The developing respiratory control system often exhibits substantial functional plasticity in response to chronic environmental perturbations (Carroll, 2003; Bavis and Mitchell, 2008). Previous studies have demonstrated long-lasting attenuation of the hypoxic ventilatory response (HVR) in mammals exposed to moderate hyperoxia (30–60% O_2) during perinatal development (*e.g.*, Ling et al., 1996; Fuller et al., 2002; Bavis et al., 2011a). This plasticity is linked to abnormal development of the carotid body, the organ which serves as the principal O_2 sensor for the respiratory control system (Bavis et al., 2013). Specifically, chronic hyperoxia inhibits postnatal growth of the carotid body (Erickson et al., 1998; Wang and Bisgard, 2005; Dmitrieff et al., 2012), causes degeneration of carotid chemoafferent neurons (Erickson et al., 1998; Chavez-Valdez et al., 2012), and diminishes carotid chemoreceptor O_2 sensitivity (Hanson et al., 1989; Bavis et al., 2011b; Kim et al., 2013). These phenotypic changes begin to appear by the fourth day of hyperoxia in rats (Donnelly et al., 2009; Bavis et al., 2011b; Dmitrieff et al., 2012), and the morphological plasticity may be permanent (Fuller et al., 2002).

Despite strong and consistent evidence for the impairment of hypoxic ventilation, the influence of chronic postnatal hyperoxia on eupneic ventilation has proven to be more variable. Several studies have examined the normoxic ventilation of adult rats that had been reared in hyperoxia (60% O_2) for the first 1–4 postnatal weeks and subsequently maintained in room air (*i.e.*, after 1–4 months of normoxic recovery). Normoxic ventilation was similar to that of age-matched control rats in some groups of rats (Wenninger et al., 2006; Bavis et al., 2007, 2011a), while the rats in other studies exhibited a mild hyperpnea and/or hyperventilation (generally <15% increase relative to controls; Ling et al., 1996; Bavis et al., 2007,

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2008). Normoxic minute ventilation also tends to be normal in adult mice reared in hyperoxia (Dauger et al., 2003; Bavis et al., 2011a), but the respiratory pattern was altered in the mice in one study (*i.e.*, slower, deeper breathing pattern following postnatal exposure to 65% O_2; Dauger et al., 2003). In contrast to adult rats and mice, Bavis et al. (2010) found that neonatal rats exposed to hyperoxia from birth exhibit markedly reduced ventilation shortly after being returned to room air; this effect was most apparent in the youngest rats studied (39% decrease relative to controls at 4 days of age (P4)) and diminished with age (*i.e.*, no longer significantly different by P13–14). The decreased ventilation in these neonatal rats reflected both lower tidal volumes (at P4, P6–7, and P13–14) and lower respiratory frequencies (at P4 only). Hyperoxia-treated rats also had reduced metabolic rates, but the reduction in O_2 consumption appeared to be too small to account for the observed reduction in ventilation (Bavis et al., 2010).

In the present study, we first sought to confirm the observation that neonatal rats exposed to chronic hyperoxia hypoventilate shortly after return to room air through measurements of ventilation, metabolic rate, and arterial O_2 saturation (as a proxy for blood gases). To further characterize this plasticity, additional measurements were made to determine whether this hypoventilation persists during a period of normoxic "recovery" and whether blood O_2 transport is enhanced to compensate for diminished ventilation (*e.g.*, increased blood O_2 carrying capacity and/or cardiac output). Finally, we investigated the mechanistic basis for the observed respiratory and metabolic plasticity. Since both the peripheral and central nervous systems contribute to eupneic ventilatory drive (Dejours, 1963; Teppema and Dahan, 2010), we evaluated the peripheral and central contributions to the observed hypoventilation. Specifically, the ventilatory response to acute O_2 inhalation was used to probe peripheral chemoreceptor involvement and an *in vitro* brainstem-spinal cord preparation was used to assess spontaneous respiratory rhythm generation. A parallel set of experiments considered whether decreased O_2 consumption reflects O_2 limitation (secondary to inadequate alveolar ventilation) versus a state of regulated hypometabolism (*e.g.*, Steiner and Branco, 2002; Mortola, 2004).

2. Methods

2.1. Experimental animals

Timed-pregnant Sprague-Dawley rats were obtained from Charles River Laboratories. For hyperoxic exposures ("Hyperoxia"), timed-pregnant dams were placed into a custom-built acrylic chamber maintained at 60% O_2, or into a Biospherix Oxycycler chamber maintained at 50–60% O_2 (for *in vitro* brainstem-spinal cord experiments, Section 2.7), 1–2 days prior to parturition; gas flow rates through the chambers were adjusted to maintain CO_2 < 0.4%. Individual Hyperoxia pups were kept with their mothers until studied between 3 and 14 days of age (*i.e.*, between P3 and P14, where the day of birth = P0); the specific ages and sample sizes studied varied by experiment and are provided in the text and/or figure legends. Age-matched control litters ("Control") were reared in identical chambers maintained at 21% O_2 (CO_2 < 0.4%). Chambers were opened briefly to remove animals for measurements and to clean cages as needed.

Control and Hyperoxia rat pups were typically studied within 15–20 min (brainstem-spinal cord experiments) or within 30–45 min (all other experiments) after removal from their respective chambers. In one experiment, Control and Hyperoxia litters were returned to the animal colony after ventilation measurements were made at P3 and subsequently housed in room air until re-studied at P4 and P6 (*i.e.*, after 1 or 3 days of normoxic recovery).

Rats were housed under a 12:12 light:dark cycle and provided food and water *ad libitum* throughout the study. All experimental procedures were approved by the Institutional Animal Care and Use Committee at Bates College, or at the University of Wisconsin (brainstem-spinal cord experiments).

2.2. Ventilation measurements

Ventilation was measured using a custom-built head-body plethysmograph chamber, as previously described (Bavis et al., 2010). The chamber was separated into head and body compartments using a flexible collar; petroleum jelly was spread around the neck to seal any gaps in the collar. Test gases (21–99% O_2, balance N_2; see below) were delivered to the head compartment at 1 l min^{-1} using a gas mixing mass flow controller (MFC-4; Sable Systems) and valves (series 840; Sierra Instruments). Respiratory-induced airflows in and out of the body compartment were detected using a pneumotach coupled to a differential pressure transducer (MLT1L and ML141; ADInstruments); the pneumotach was calibrated prior to placing each animal into the plethysmograph by injecting 0.5 ml of air into the sealed body compartment. Respiratory airflows were recorded to a computer at a sampling rate of 1000 Hz, integrated, and digitally filtered (high-pass, 0.1 Hz) to obtain respiratory volumes (PowerLab 8SP and LabChart 6 or 7 software; ADInstruments). Air temperature in the body compartment was monitored continuously with a T-type thermocouple probe (Physitemp Instruments) and maintained at 32–34 °C (*i.e.*, within the thermoneutral zone of neonatal rats; Malik and Fewell, 2003) with the aid of an incubator.

Two different experimental protocols were used. In the first set of experiments, ventilation was measured in Control rats (from 12 litters) and Hyperoxia rats (from 9 litters) at P4, P6-7, and P13-14; individuals were studied only once. After being sealed into the plethysmograph, rats were given 10–15 min to adjust to the chamber before commencing the ventilation measurements. During this adjustment period, rats were exposed to either 21% O_2 (approximately half of the Control group) or 60% O_2 (remainder of the Control group and all Hyperoxia rats); subsequent analysis revealed that inspired O_2 levels during this adjustment period had no effect on the ventilation of Control rats, so data for all Control rats were pooled. Once the rat appeared calm (based on stability of the respiratory pattern), respiratory airflows were recorded during exposures to 60% O_2 (10 min), 21% O_2 (10 min), and 99% O_2 (1 min), presented in that order; changeover of gases in the plethysmograph head chamber from 21% O_2 to 99% O_2 took approximately 30 s. Ventilatory parameters were analyzed on a breath-by-breath basis and averaged over 45–60 s at the end of the 60% O_2 and 21% O_2 exposures and over the final 10–15 s of the 99% O_2 exposure, excluding movement artifacts and sighs; usable data were obtained for only a subset of rats during the 99% O_2 trials because of excessive movement artifacts during the final 10–15 s for some individuals (compare sample sizes in Figs. 1 and 2).

In a separate set of experiments, ventilation was measured for Control (from 2 litters) and Hyperoxia (from 2 litters) rats at P3, and then re-measured for the same individuals at P4 and P6 (*i.e.*, after 1 and 3 days of recovery in room air). After being sealed into the plethysmograph chamber, rats were given 10–15 min to adjust to the chamber while breathing 21% O_2 before commencing the ventilation measurements. Ventilation was then recorded for approximately 7 min in 21% O_2; ventilatory parameters were analyzed on a breath-by-breath basis and averaged over 45–60 s at the end of the exposure, excluding movement artifacts and sighs.

2.3. Metabolism and body temperature measurements

O_2 consumption and CO_2 production were measured by open-system respirometry. Rats were sealed into clear plastic boxes