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Impaired hypoxic ventilatory response following neonatal sustained and subsequent chronic intermittent hypoxia in rats

C.A. Mayer, J. Ao, J.M. Di Fiore, R.J. Martin, P.M. MacFarlane*

Department of Pediatrics, Case Western Reserve University, Rainbow Babies & Children's Hospital, Cleveland, OH 44106, USA

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ABSTRACT

Neonatal chronic intermittent hypoxia (CIH) enhances the ventilatory sensitivity to acute hypoxia (acute hypoxic ventilatory response, HVR), whereas sustained hypoxia (SH) can have the opposite effect. Therefore, we investigated whether neonatal rats pre-treated with SH prior to CIH exhibit a modified HVR. Rat pups were exposed to CIH (5% O_2/5 min, 8 h/day) between 6 and 15 days of postnatal age (P6-15) after pre-treatment with either normoxia or SH (11% O_2; P1-5). Using whole-body plethysmography, the acute (5 min, 10% O_2) HVR at P16 (1 day post-CIH) was unchanged following CIH (67.9 ± 6.7% above baseline) and also SH (58.8 ± 10.5%) compared to age-matched normoxic rats (54.7 ± 6.3%). In contrast, the HVR was attenuated (16.5 ± 6.0%) in CIH exposed rats pre-treated with SH. These data suggest that while neonatal SH and CIH alone have little effect on the magnitude of the acute HVR, their combined effects impose a synergistic disturbance to postnatal development of the HVR. These data could provide important insight into the consequences of not maintaining adequate levels of oxygen saturation during the early neonatal period, especially in vulnerable preterm infants susceptible to frequent bouts of hypoxemic events (CIH) that are commonly associated with apnea of prematurity.

1. Introduction

Supplemental oxygen (O_2) therapy is frequently used in the intensive care of preterm infants to protect against hypoxemia. Recent clinical trials (see the SUPPORT Study, Carlo et al., 2010), have been designed to mitigate the progression of O_2-related morbidities such as retinopathy of prematurity (ROP) by targeting lower levels of O_2 saturation (SaO_2) in the first 2 months of intensive care. However, the preterm infant also experiences periods of O_2 de-saturation frequently associated with apnea (apnea of prematurity, AOP), which increase profoundly after the first week of life (Di Fiore et al., 2010; Martin et al., 2011). Targeting a lower level of O_2 saturation has resulted in a postnatal profile of O_2 exposure experienced by the preterm infant consisting of sustained hypoxemia (SH) with subsequent superimposed chronic intermittent hypoxia (CIH) associated with AOP. Since animal studies typically investigate the separate effects of SH and CIH on the postnatal development of the respiratory control system, we devised a rodent model that combines SH treatment prior to CIH to more closely mimic some of the O_2 profile characteristics experienced by preterm infants.

Early life experiences during sensitive stages of development can leave long-lasting effects on the respiratory neural control system (Bavis and Mitchell, 2008). Neonatal SH and CIH exposure are hallmarks of clinically relevant conditions that are used to assess the development of the acute hypoxic ventilatory response (HVR). Neonatal CIH, a paradigm often used to mimic AOP, has been shown to elicit a variable effect on the HVR. In some cases, the CIH has been shown to have no influence or attenuate (Reeves et al., 2006) the HVR whereas others have shown it is enhanced compared to age-matched rats raised in normoxia (Peng et al., 2004; Reeves and Gozal, 2006). The discrepancies in these effects may be related to several factors that include the duration, intensity of exposure, and method of normalizing the changes in ventilation during acute hypoxia (Reeves and Gozal, 2005). An enhanced response following CIH is consistent with the positive correlation that exists between the incidence of apneic events and the magnitude of the HVR observed in preterm infants (Nock et al., 2004). The effects of neonatal CIH seem to persist into adulthood and can be partly explained by augmented carotid body chemoreceptor sensitivity (*sensitization*) to acute hypoxia (Pawar et al., 2008). On the other hand, neonatal SH attenuates the HVR, resulting largely from impaired carotid body chemosensitivity (Eden and Hanson, 1987; Hanson et al., 1989; Sladek et al., 1993; Wyatt et al., 1995). Whether a blunted HVR is adaptive or maladaptive is largely unknown, although surgical denervation of the carotid bodies profoundly disturbed the arousal response to acute hypoxia in sleeping lambs (Bureau et al., 1985) and dogs (Bowes et al., 1981), indicating

* Corresponding author at: Department of Pediatrics, Case Western Reserve University, Rainbow Babies & Children's Hospital, 11100 Euclid Avenue, Cleveland, OH 44106-6010, USA. Tel.: +1 216 368 4628.
E-mail address: pmm71@case.edu (P.M. MacFarlane).

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functional carotid bodies may be critical for mounting an appropriate protective response to hypoxia that accumulates during an apneic event (MacFarlane et al., 2012). Furthermore, a previous study revealed increased mortality in rat pups if SH began prior to 5 days of age (Mortola, 2001), suggesting that very early windows of development may be uniquely sensitive to sustained periods of de-saturation in a way that could lead to potentially debilitating disturbances in the development of the respiratory neural control system.

In a recent clinical trial (Carlo et al., 2010) preterm infants of 24–28 weeks gestation were randomized to a "*low*" (85–89%) or "*high*" (91–95%) O_2 saturation target range in an attempt to reduce the incidence of bronchopulmonary dysplasia and ROP. In a subcohort of infants both target groups were shown to have a low incidence of hypoxic events during the first week of life followed by a profound increase over the following weeks (Di Fiore et al., 2012). Thus, since the incidence of desaturation events was very minimal in the early postnatal period, then one of the primary distinctions between the "*high*" and "*low*" target groups was that the latter experience a period of continuous hypoxemia during the first postnatal week prior to developing subsequent CIH. While the "*low*" target group had a lower incidence of retinopathy, a disconcerting finding was an increase in infant mortality compared to the "*high*" target group (Stenson et al., 2011). The cause for the infant mortality is unknown, although these findings indicate a possible lethal effect of SH and subsequent CIH during uniquely sensitive windows of development. Therefore, the purpose of this study was to assess the possibility of a disturbance in the respiratory neural control system initiated by SH and subsequent CIH exposure during the first few weeks of life. We hypothesized that neonatal SH followed by CIH could lead to a modification in the respiratory neural control system, manifest as an attenuated acute HVR.

2. Experimental procedures

Time-pregnant Lewis rats were purchased from a commercial vendor (Charles River, colony PO6) and were later observed to give birth in the animal facility of the institution; experiments were performed on 16 day old male rats. All procedures were carried out in accordance with the National Institute of Health (NIH) guidelines for care and use of laboratory animals and were approved by the Animal Care and Use Committee at Case Western Reserve University.

2.1. Hypoxia exposures

Following the day of birth (P0), the dam (and her pups) were assigned to one of 4 groups that received one of the following experimental protocols until the pups were 16 days of age: normoxic (**Nx**) raised rat pups (12 pups from 4 litters); **CIH** only treated rats (11 pups from 3 litters): pups raised in normoxia (Nx) for 5 days, followed by 10 days of CIH; **SH + CIH** treated rats (10 pups from 3 litters): pups raised in sustained hypoxia (**SH**, 11% O_2) for 5 days, followed by 10 days of CIH; SH treated rats (10 pups from 3 litters): pups raised in SH for 5 days, followed by 10 days of Nx. SH was achieved by placing the mother and pups inside a plexiglas chamber (30 cm × 50 cm × 28 cm) connected to adjustable rotameters for mixing air and nitrogen (N_2). Oxygen levels were monitored (TED 60T, Teledyne Analytical Instruments; CA, USA) and adjusted if necessary to maintain sustained hypoxia (~11% O_2). Airflow through the chambers was maintained at ~3 L/min and carbon dioxide (CO_2) levels in the airflow exiting the chambers was monitored to ensure flow was adequate to prevent CO_2 accumulation. Sustained hypoxia exposure lasted 24 h/day for 5 consecutive days. At the end of the 5th day of exposure, the rats were then raised for a further 10 days in either normoxia or CIH, according to the groups outlined above.

Chronic intermittent hypoxia consisted of purging air inside custom-made chambers (53 cm × 58 cm × 23 cm) using N_2 until the O_2 levels reached 5% O_2 at which time air was again purged into the chambers to return to room air. Switching between air and N_2 flow through the chamber was achieved using time-controlled solenoid valves so that each hypoxic nadir (i.e. 5% O_2) occurred every 5 min (Fig. 1A), 8 h/day for 10 days. CIH exposure occurred during the nocturnal period of their light cycle (between 9am-5pm). Cages, water and food were replaced every 3 days. Normoxic rats received room air for the same time period.

2.2. Plethysmography and rates of metabolism

The day after (P16) the rat pups received their last exposure (P15), the ventilatory responses to acute hypoxia and hypercapnia were assessed using whole-body plethysmography. The pups were removed from the litter and placed inside a custom-made perspex plethysmograph chamber (Fig. 1B; volume = 265 ml) sealed with rubber plungers at each end. A platform was placed inside the chamber to minimize dead space and to allow the rat to rest comfortably. Airflow through the chambers allowed for continuous measurement of expired O_2 and CO_2 concentrations ($F_{E_{O_2}}$, $F_{E_{CO_2}}$), whereas 3-way stopcocks positioned up- and downstream of the chamber allowed the airflow to momentarily bypass the chamber for measurement of inspired O_2 and CO_2 concentrations ($F_{I_{O_2}}$, $F_{I_{CO_2}}$). Ventilation was measured during the time the chamber was sealed. The chamber was placed on a heated pad to maintain constant internal temperature. Temperature inside the chamber was maintained (~28 °C) by adjusting a water bath (Isotemp 3013S, Fisher Scientific; PA, USA) that circulated water to a heat pad positioned underneath the plethysmograph. Airflow through the chamber was held constant at 450 ml/min using a mass flow controller (Aalborg, 0–2 L/min; NY, USA), and hypoxia (12% and 10% O_2) was administered using a gas mixer (Bird Blender 2003, Pneumatic Services Inc.; FL, USA). Prior to each experiment, the chamber was assessed for adequate seal by observing stability of the square pressure change following injection of a calibration volume (50 μl) using a glass micro-syringe (Hamilton, Harvard Apparatus; MA, USA). The same injection volume was used later for calibration of tidal volume changes associated with breathing (see below). Rectal temperature was monitored continuously throughout the experiment with a fine temperature thermocouple (Physitemp; NJ, USA), which was held securely in place with tape adhered to the base of the tail. O_2 and CO_2 in the gas that passed through the chamber was also measured continuously using appropriate gas analyzers (ADInstruments, Gas Analyzer ML206; CO, USA).

The plethysmograph enabled accurate measurement of the rates of ventilation ($\dot{V}_E$) and metabolism (oxygen consumption, $\dot{V}_{O_2}$; carbon dioxide production, $\dot{V}_{CO_2}$) during baseline, hypoxia, and hypercapnia. Rats were allowed ~25 min to acclimatize to the chamber before receiving graded hypoxia, which consisted of 12% O_2 (5 min), a recovery period in normoxia (5 min), 10% O_2 (5 min), followed by hypercapnia (5% CO_2, 5 min). Ventilation was measured when the chambers were sealed during the last 30 s of exposure. Chambers were sealed by turning stopcocks upstream and downstream of the plethysmograph to bypass airflow, which was also used to determine the incurrent fractional concentrations of O_2 and CO_2 for determining the rates of metabolism (see below). The corresponding pressure signal associated with breathing during the time the chamber was sealed and calibrated for volume using a glass syringe (50 μl injection) for calculating tidal volume using