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Potential of the hypoxic ventilatory response by one day of hyperoxia in neonatal rats

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Abstract

The O₂ sensitivity of the neonatal rat carotid body is increased after one day in moderate hyperoxia (60% O₂) (Donnelly et al., *Respir. Physiol. Neurobiol.* 168: 189–197, 2009). We investigated whether this enhanced peripheral chemosensitivity increases the hypoxic ventilatory response (HVR) and tested the hypothesis that this plasticity is mediated by the superoxide anion. Neonatal rats (7 d old) were injected with saline or MnTMPyP, a superoxide scavenger, and placed into 60% O₂ for 23–28 h. Baseline ventilation was reduced and the acute HVR (12% O₂) was enhanced in hyperoxia-treated rats relative to age-matched controls; MnTMPyP did not block these effects. An additional group of rats was studied after only 30 min in 60% O₂. This shorter exposure had no effect on normoxic ventilation or the HVR. We conclude that 1 d, but not 30 min, of 60% O₂ augments the HVR of neonatal rats and that production of the superoxide anion does not contribute to this plasticity.

Keywords

control of breathing; hypoxia; plasticity; reactive oxygen species; superoxide anion

1. Introduction

Chronic hyperoxia (30–60% O₂) for the first 1–4 postnatal weeks causes a long-lasting reduction in the hypoxic ventilatory response (HVR) in rats, primarily by impairing carotid body development (Ling et al., 1997; Bavis, 2005). In addition to reducing carotid body size and the number of chemoafferent axons in the carotid sinus nerve (Erickson et al., 1998; Wang & Bisgard, 2005), hyperoxia progressively diminishes O₂ sensitivity of the carotid body chemoreceptors (Donnelly et al., 2005, 2009). In a recent study by Donnelly and colleagues (2009), neonatal rats were exposed to 60% O₂ for up to two weeks beginning at 7 days of age (P7). Carotid body O₂ sensitivity was reduced by P12 (i.e., after 5 d in hyperoxia) and remained low throughout the remainder of the hyperoxic exposure. There was, however, an unexpected *increase* in O₂ sensitivity at P8 (i.e., after 1 d in hyperoxia). Specifically, *in vitro* single-unit chemoafferent activity and glomus cell intracellular Ca²⁺ responses to hypoxia were greatly enhanced upon exposure to severe hypoxia (Donnelly et al., 2009). The primary objective of the present study, therefore, was to determine whether

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the enhanced carotid body response to hypoxia following a 1 d exposure to moderate hyperoxia would cause a corresponding increase in the acute HVR in the intact animal.

The cellular pathways responsible for hyperoxia-induced potentiation of carotid body sensitivity are not known. Hyperoxia has a variety of excitatory effects on respiratory control in adult mammals, including hyperoxic hyperventilation (i.e., increased ventilation during a sustained exposure to hyperoxia) (Cragg et al., 1986, Becker et al., 1996), increased excitability of brainstem neurons linked to respiratory control (Mulkey et al., 2003), and glutamate- and nitric oxide (NO)-dependent potentiation of the HVR (Honda et al., 1996; Gozal, 1998; Pokorski et al., 2005). In each of these cases, however, the excitatory effects of hyperoxia (often $\geq 100\%$ inspired O_2) are believed to occur within the CNS, not the carotid body (Gozal, 1998; Dean et al., 2004). Interestingly, Peng and colleagues (2004) found that neonatal rats (P2) previously exposed to intermittent hypoxia (15 s of 5% O_2 separated by 5 min of 21% O_2) for 16 hours exhibit greater carotid body O_2 sensitivity (i.e., increased single-unit chemoafferent responses to hypoxia *in vitro*) and augmented HVR. Longer periods of intermittent hypoxia (10 d) have a similar effect on carotid body O_2 sensitivity in neonatal rats (Pawar et al., 2008, 2009), and these effects are completely blocked by daily administration of the antioxidant drug manganese (III) tetrakis (1-methyl-4-pyridyl) porphyrin pentachloride (MnTMPyP), a potent, membrane-permeable superoxide anion ($O_2^{\bullet-}$) scavenger (Pawar et al., 2009). Like intermittent hypoxia, hyperoxia increases the production of $O_2^{\bullet-}$ and other reactive oxygen species (Jamieson et al., 1986; Fabian et al., 2004; D'Agostino et al., 2007), even at moderate inspired O_2 levels (e.g., 50–70% O_2 ; Brueckl et al., 2006). Thus, increased $O_2^{\bullet-}$ production is one potential mechanism by which hyperoxia might enhance carotid body O_2 sensitivity and the acute HVR. To test this hypothesis, we attempted to block hyperoxia-induced potentiation of the HVR using MnTMPyP.

2. Methods

2.1 Experimental design

Experiments were conducted on neonatal Sprague-Dawley rats (SAS SD, Charles River Laboratories, Wilmington, MA USA). Rats were born and raised with their mothers under standard conditions (12:12 light cycle, food and water *ad libitum*) until studied at P7-P8. All experimental procedures were approved by the Animal Care and Use Committee at Bates College.

In the first experiment, individual P7 rats (either sex) from ten litters received intraperitoneal injections of the antioxidant drug MnTMPyP pentachloride (5 mg ml^{-1} in saline; Alexis Biochemicals, San Diego, CA USA) or an equal volume of vehicle (0.9% saline; Phoenix Pharmaceutical, St. Joseph, MO USA). Each pup was injected with 0.01 ml per 10 g body mass (i.e., 0.01 ml for 0–10.0 g, 0.02 ml for 10.1–20.0 g, etc.); thus, MnTMPyP pups received 5–10 mg kg^{-1} of the drug depending on their mass, equal to or exceeding the effective dosage used for rats in previous studies (e.g., Pawar et al., 2009). Five of these litters (“Hyperoxia”) were then placed into an environmental chamber maintained at 60% O_2 ($CO_2 < 0.4\%$) for approximately 1 d (23–28 h). The remaining five litters (“Control”) were placed into an environmental chamber maintained at 21% O_2 ($CO_2 < 0.4\%$) for the same time period. An additional group of rats was prepared at a later date to determine the effect of a supplemental dose of MnTMPyP. All pups in this group were injected with MnTMPyP and placed into environmental chambers flushed with 60% O_2 (2 litters) or 21% O_2 (3 litters) as described above, but individuals received a second injection of the drug (5–10 mg kg^{-1} , i.p.) 12 h after the initial dose was administered; in the following sections, rats that received this booster injection are indicated by “MnTMPyP $\times 2$ ”.

In a second experiment, three additional litters of rats were used to study the respiratory effects of a shorter hyperoxic exposure; no drugs were administered to these rats. P7–8 rats (either sex) from these litters were placed individually into a 600 ml acrylic chamber flushed with gas containing either 60% O₂ (“Hyperoxia”) or 21% O₂ (“Control”) for 30 min (flow rate = 2 l min⁻¹). Since the rats were separated from the rest of their litter during this period, a water bath was used to warm the chamber to ~34°C (i.e., within the neonatal rat thermoneutral zone; Malik & Fewell, 2003) throughout the exposure.

Individual rats were removed from their respective chambers after 23–28 h (experiment 1) or 30 min (experiment 2) and immediately transferred to a plethysmograph for ventilation measurements. The number of individuals studied in each treatment group ranged from 10–18; specific sample sizes are reported in the figure legends.

2.2. Ventilation measurements

Ventilation measurements were made using a customized head-body plethysmograph. The plethysmograph consisted of two acrylic cylindrical chambers: a head chamber (4.75 cm ID; ~70 ml) through which test gases flowed and a body chamber (3.1 cm ID; internal volume was adjusted using modeling clay to limit the backward movement of the animal). A flexible collar made from layers of latex film (~0.15 mm thick) and Parafilm (Pechiney Plastic Packaging, Menasha, WI USA) was fitted around the neck of the rat, thereby isolating the head and body compartments; a layer of petroleum jelly was applied around the rat’s neck to prevent gas leaks. The body chamber contained a T-type thermocouple probe (IT-18, Physitemp Instruments, Clifton, NJ USA) to monitor air temperature as well as ports connected to a pneumotach (MLT1L, ADInstruments, Colorado Springs, CO USA) and a calibration syringe. The pneumotach was connected to a differential pressure transducer (ML141, ADInstruments, Colorado Springs, CO USA) to monitor respiratory airflows; the pneumotach was calibrated at the start of each experiment using a 0.5 ml injection of air. Respiratory airflows and chamber temperature were recorded continuously to a computer at a sampling rate of 1000 Hz (PowerLab 8SP and LabChart 6 software, ADInstruments, Colorado Springs, CO USA). Respiratory airflows were integrated and digitally filtered (high-pass, 0.1 Hz) to obtain respiratory volumes.

The plethysmograph was situated in an incubator so that the air temperature in the body chamber could be maintained at 32–34°C (i.e., within the thermoneutral zone of neonatal rats; Malik & Fewell, 2003). Gas flow through the head chamber was set at 1500 ml/min using a gas mixing mass flow controller (MFC-4; Sable Systems, Las Vegas, NV USA) and valves (series 840; Sierra Instruments, Monterey, CA USA). After being weighed and sealed into the plethysmograph, the rat was exposed to 21% O₂ (balance N₂) for at least 10 min. Once the rat appeared calm (based on stability of the respiratory pattern), respiratory airflows were recorded under baseline conditions (21% O₂) and during an 8-min exposure to hypoxia (12% O₂).

2.3. Data Analysis

A breath-by-breath analysis of respiratory variables (tidal volume, respiratory frequency, and minute ventilation) was made for 30–45 s during baseline and for approximately 10 s at the end of the first minute of hypoxia (early phase of the HVR) and at the end of the eighth minute of hypoxia (late phase of the HVR), excluding obvious movement artifacts, sighs or vocalizations. The early phase of the HVR is generally thought to reflect activation of the peripheral chemoreflex, whereas changes in ventilation during longer exposures (i.e., late HVR) may be influenced by central respiratory depression and changes in metabolism (Teppema & Dahan, 2010). If no differences were detected between treatment groups during the initial minute of hypoxia, respiratory variables were also calculated for the second

minute of hypoxia (see Results sections 3.1 and 3.2). Hypoxic responses were expressed as a percentage increase from baseline.

In Experiment 1, statistical comparisons among groups were made using two-way analysis of variance [factor 1: hyperoxia treatment (Control, Hyperoxia); factor 2: drug treatment (Saline, MnTMPyP)]. Control and Hyperoxia groups that received supplemental MnTMPyP (i.e., MnTMPyP $\times$ 2) were compared using independent samples t-tests. In Experiment 2 (30 min gas exposures), Control and Hyperoxia groups were compared using independent samples t-tests. Differences were considered significant at $P < 0.05$. All statistical tests were run using SigmaStat 3.11 (Systat Software, San Jose, CA, USA).

3. Results

3.1 Experiment 1: 1 d exposure to hyperoxia

Rat pups exposed to 60% O₂ for 23–28 h exhibited lower resting ventilation while breathing room air (21% O₂) than their age-matched controls (main effect for hyperoxia, $P < 0.001$), and this did not vary with drug treatment (hyperoxia $\times$ drug, $P = 0.14$) (Fig. 1A). Averaged across drug treatment groups (i.e., Saline and MnTMPyP), the minute ventilation for Hyperoxia rats was 25% lower than that of Control rats. This effect was primarily explained by a lower tidal volume in Hyperoxia rats (main effect for hyperoxia, $P = 0.002$) (Fig. 1B), but there was also a trend toward lower respiratory frequency ($P = 0.06$) (Fig. 1C). Independent of O₂ levels during the previous day, rats receiving MnTMPyP had a slightly lower baseline respiratory frequency compared to their saline-injected counterparts (main effect for drug, $P = 0.02$) (Fig. 1C); no effects of MnTMPyP were detected for ventilation or tidal volume (i.e., drug and hyperoxia $\times$ drug, $P > 0.05$).

Pretreatment with hyperoxia augmented both the early and late components of the acute HVR of neonatal rats (Fig. 2A), and MnTMPyP did not diminish this effect (hyperoxia $\times$ drug, $P = 0.30$ and $P = 0.54$ for early and late HVR, respectively). The increases in minute ventilation during 12% O₂ were greater in Hyperoxia rats than in Controls during the early and late HVR (main effect for hyperoxia, $P = 0.002$ and $P = 0.04$, respectively). Due to the lower baseline ventilation of Hyperoxia rats, however, the absolute value for minute ventilation achieved in hypoxia did not differ significantly between Hyperoxia and Control rats ($P = 0.10$ and $P = 0.08$ for early and late HVR, respectively). The greater HVR of Hyperoxia rats (as a percentage of baseline) primarily reflected an augmented tidal volume response (main effect for hyperoxia, $P = 0.001$ and $P = 0.02$ for early and late HVR, respectively) (Fig. 2B). Hyperoxia had no effect on the frequency response to acute hypoxia ($P = 0.87$ and $P = 0.41$ for early and late HVR, respectively) (Fig. 2C).

Although MnTMPyP treatment did not influence the hyperoxia-induced potentiation of the HVR (i.e., no significant treatment $\times$ drug interaction), this drug altered the acute HVR independent of the treatment group (i.e., main effect for drug). Specifically, rats that received a single dose of MnTMPyP tended to have reduced HVR compared to saline-injected rats (Fig. 2A), but this decrease was only significant during the late phase of the HVR (main effect for drug, $P = 0.11$ and $P = 0.03$ for early and late HVR, respectively). This reflected smaller increases in tidal volume during both the early and late phases of the HVR (main effect for drug, $P = 0.04$ and $P = 0.003$, respectively) (Fig. 2B). MnTMPyP had no effect on the frequency response to acute hypoxia ($P = 0.76$ and $P = 0.49$ for early and late HVR, respectively) (Fig. 2C).

To determine whether an additional dose of MnTMPyP would block the respiratory effects of the 1 d hyperoxia treatment, a subset of Control and Hyperoxia rats was administered a booster injection of the drug 12 h after the first injection (i.e., MnTMPyP $\times$ 2). Baseline tidal

volume was significantly reduced in Hyperoxia rats (0.55 ± 0.03 vs. 0.66 ± 0.04 ml 100g^{-1} in Control; $P=0.01$) with no difference in respiratory frequency ($P=0.99$). As a result, there was a marginally significant trend for Hyperoxia rats to exhibit lower minute ventilation under baseline conditions ($P=0.07$) (Fig. 3A). There was also a non-significant trend for the HVR to be greater during the first minute of hypoxia in Hyperoxia rats compared to their Control group ($P=0.18$) (Fig. 3B, left panel). Although we initially planned to use minute 1 to represent the early phase of the HVR, the early HVR appears to last several minutes in neonatal rats (Bavis et al., 2010). Therefore, to ensure that we did not overlook changes to the early HVR in this smaller sample of rats, we subsequently examined the HVR during the second minute of hypoxia as well. In this *post hoc* analysis, the early HVR of Hyperoxia rats was approximately double that of the Controls ($P<0.001$) (Fig. 3B, right panel). The late HVR was also significantly greater in Hyperoxia rats ($P<0.001$) (Fig. 3C). Accordingly, a second dose of MnTMPyP did not prevent hyperoxia-induced potentiation of the HVR.

3.2 Experiment 2: 30 min exposure to hyperoxia

The results from Experiment 1 indicate that 1 d of hyperoxia is sufficient to alter respiratory control in neonatal rats, but this raises the question of whether prolonged hyperoxia is necessary to elicit this plasticity. To determine whether an acute hyperoxic exposure might have the same effect, rats in this second experiment were exposed to only 30 min of 60% O_2 (or 21% O_2 as a control). Baseline minute ventilation did not differ between Hyperoxia and Control rats ($P=0.34$) (Fig. 4A), nor did tidal volume or respiratory frequency (data not shown). There also were no differences detected between treatment groups for the early HVR (minute 1: $P=0.46$; minute 2: $P=0.49$) or late HVR ($P=0.32$) (Fig. 4B, C).

4. Discussion

This study confirmed that a relatively brief (23–28 h) period of moderate hyperoxia induces plasticity in the respiratory control system of neonatal rats. Specifically, rat pups exposed to approximately 1 d of 60% O_2 exhibited a lower tidal volume following return to room air and a greater ventilatory response to 12% O_2 . The augmented HVR is consistent with the enhanced carotid body O_2 sensitivity previously reported for rat pups exposed to a similar duration of hyperoxia (Donnelly et al., 2009). Ventilation measurements were made shortly after removal from hyperoxia (typically 10–30 min in normoxia prior to the onset of hypoxia, including time to establish baseline breathing), thus it is not known how long this plasticity persists. It is clear, however, that acute hyperoxic exposures (≤ 30 min) do not produce comparable changes to the respiratory control of neonatal rats. Finally, contrary to our hypothesis, the superoxide scavenging drug MnTMPyP did not prevent this plasticity.

4.1 Reduced normoxic ventilation following 1 d of hyperoxia

After 1 d in 60% O_2 , neonatal rats exhibited reduced tidal volumes while breathing room air and, as a result, generally exhibited reduced minute ventilation as well. The reduction in minute ventilation was not quite significant in the MnTMPyP $\times 2$ group ($P=0.07$), but this likely reflects the relatively small sample sizes for this comparison ($n=12-13$) rather than an effect of the drug *per se* since: (1) hyperoxia-treated rats had significantly lower tidal volumes and normal respiratory frequencies compared to their control group and (2) the average minute ventilation was 18% lower in hyperoxia-treated rats in the MnTMPyP $\times 2$ group, similar in magnitude to the 25% reduction in minute ventilation observed in hyperoxia-treated rats that received only a single injection of saline or MnTMPyP. Therefore, these data do not indicate a major role for $\text{O}_2^{\bullet-}$ in the observed changes to eupneic breathing.

We recently reported reduced tidal volume and minute ventilation in neonatal rats exposed to 4–7 d of 60% O₂ from birth (Bavis et al., 2010). It therefore appears that 1 d and 7 d hyperoxic exposures have qualitatively similar effects on normoxic breathing. In the earlier study, it was found that chronic hyperoxia also decreased normoxic O₂ consumption (Bavis et al., 2010). Accordingly, lower baseline ventilation may reflect, at least in part, reduced metabolic demands in hyperoxia-treated rats. It is also possible that hyperoxia-treated rats experience diminished tonic drive from peripheral chemoreceptors. Although not specifically reported in the paper by Donnelly and colleagues (2009), baseline carotid chemoafferent neuron discharge rate was reduced in P8 rats after a 1-d exposure to hyperoxia (0.2 ± 0.1 vs. 1.3 ± 0.4 Hz in age-matched controls; D.F. Donnelly & R.W. Bavis, unpublished data). Chronic hyperoxia also causes degeneration of carotid chemoafferent neurons (Erickson et al., 1998), but whether this occurs in as little as one day remains to be determined.

4.2 Augmented HVR following 1 d of hyperoxia

The acute HVR was greater in neonatal rats subjected to 1 d of 60% O₂. This effect was apparent within the first 1–2 minutes of hypoxia (i.e., the early HVR), indicating increased activation of the peripheral chemoreflex; metabolism was not measured in this study, but it is unlikely that altered metabolic responses to hypoxia would influence the early phase of the HVR. Although we cannot rule out an additional CNS contribution to this plasticity, the most likely explanation for the augmented HVR is enhanced sensitivity of the carotid chemoreceptors themselves: *in vitro* carotid chemoafferent and glomus cell intracellular Ca²⁺ responses to hypoxia are enhanced after a similar duration of hyperoxia (Donnelly et al., 2009). Longer durations (>4 d) of hyperoxia are known to diminish carotid body (Bisgard et al., 2003; Donnelly et al., 2005, 2009) and ventilatory responses to hypoxia (Ling et al., 1997; Bavis et al., 2010) which suggests that this excitatory plasticity is only transiently expressed and/or that it is subsequently obscured by other (inhibitory) forms of plasticity that develop more slowly.

Given the qualitatively similar effects of chronic intermittent hypoxia and 1 d of hyperoxia (i.e., increased carotid body and ventilatory responses to hypoxia) and the fact that both conditions stimulate the production of reactive oxygen species, we hypothesized that these forms of plasticity would share the same mechanistic pathways. In contrast to chronic intermittent hypoxia, however, there was no evidence that increased production of O₂^{•−} plays a significant role in the potentiation of the HVR (and presumably carotid body O₂ sensitivity) after a 1 d hyperoxic exposure in neonatal rats. A single, daily injection of MnTMPyP is sufficient to abolish carotid body plasticity during chronic intermittent hypoxia in neonatal (Pawar et al., 2009) and adult (Peng & Prabhakar, 2004) rats, but this treatment did not diminish the effects of hyperoxia on the acute HVR; this was also the case when rats received a supplemental dose of MnTMPyP halfway through the hyperoxic exposure. Importantly, systemic injection of MnTMPyP blocks O₂^{•−} production in both peripheral and CNS tissues (e.g., Peng et al., 2006; Sharma et al., 2009), so it is unlikely that O₂^{•−} contributes directly to any (as of yet undiscovered) central component to this plasticity either. However, we cannot completely exclude a role for reactive oxygen species, other than O₂^{•−}, or reactive nitrogen species in this plasticity without administering a variety of antioxidant drugs.

It has been suggested that acute hyperoxia can enhance the acute HVR in adult mammals through an NO-dependent mechanism (Gozal, 1998; Pokorski et al., 2005). Specifically, conscious (Gozal, 1998) and anesthetized (Pokorski et al., 2005) adult rats exhibit augmented HVR after breathing 100% O₂ for only 10–15 minutes. Although we did not observe a change in the HVR of neonatal rats exposed to 30 min of 60% O₂, it is possible that longer exposures are required to initiate this plasticity in neonates and/or when lower

inspired O₂ levels are used (i.e., 60 vs. 100% O₂). Gozal (1998) reported that selective inhibition of neuronal nitric oxide synthase (nNOS) completely abolishes the effect of 100% O₂ on the HVR in adult rats. Since NO generally inhibits carotid body responses to hypoxia (Lahiri et al., 2006) but exerts excitatory effects on the HVR centrally, Gozal (1998) postulated that hyperoxia potentiates the acute HVR by increasing NO production in the brainstem. While we cannot rule out central effects of hyperoxia on the HVR of neonatal rats, Gozal's model does not explain the increased carotid body O₂ sensitivity (Donnelly et al., 2009) that almost certainly contributes to the hyperoxia-induced potentiation of the HVR observed in the present study. Interestingly, Pokorski and colleagues (2005) found that inhibition of nNOS or combined inhibition of nNOS and glutamate receptors diminished, but did not eliminate, the effects of 100% O₂ exposure on the HVR of anesthetized, adult rats; the residual enhancement of the HVR could originate from peripheral chemoreceptors in this case. Likewise, it would be interesting to know whether there is a central, NO-dependent component to the augmented HVR of neonatal rats after 1 d hyperoxia.

4.3 MnTMPyP and the acute HVR

Although MnTMPyP did not prevent hyperoxia-induced potentiation of the acute HVR, this drug did have an effect on respiratory control. MnTMPyP reduced the HVR in both normoxia-treated and hyperoxia-treated rats (i.e., independent of the level of O₂ experienced during the preceding 23–28 h), as shown by the significant main effect for the drug treatment in Figures 2A and 2B. Interestingly, a similar effect has been noted in anesthetized, adult rats. Phrenic nerve responses to acute hypoxia tended to be reduced in adult rats after acute administration of 5 or 10 mg kg⁻¹ doses of MnTMPyP (MacFarlane & Mitchell, 2008, their Fig. 2). Although this effect was not statistically significant when groups receiving the two doses were considered separately (n=8 per group), the reduction is significant when these rats are pooled to increase statistical power [77±9% increase in phrenic burst amplitude for MnTMPyP-treated rats vs. 120±29% increase for vehicle-treated controls, *P*=0.04 (independent samples t-test); P.M. MacFarlane & G.S. Mitchell, personal communication]. Together, these observations suggest a role for O₂^{•-} in the acute HVR of normal, untreated rats.

The role of ROS in O₂ sensing at the carotid body continues to be debated (Lahiri et al., 2006; Gonzalez et al., 2007), so it is not clear whether MnTMPyP could directly interfere with this mechanism. Alternatively, Gonzalez and colleagues (2007) propose that superoxide dismutase mimetics such as MnTMPyP may increase NO levels (by limiting the reaction of O₂^{•-} and NO to form peroxynitrite), thereby inhibiting carotid body activity. However, there is no evidence that repeated, systemic administration of MnTMPyP depresses subsequent *in vitro* carotid body activity in neonatal rats (Pawar et al., 2009). It is also possible that O₂^{•-} or other reactive oxygen species normally contribute to regulation of the acute HVR downstream of the carotid chemoafferent neurons, or perhaps at other peripheral/central chemoreceptors (e.g., Dean, 2010).

5. Conclusions

We have shown that a 1 d exposure to moderate hyperoxia augments the acute HVR in neonatal rats. This is opposite of the effect produced by prolonged developmental hyperoxia (which blunts the HVR; Ling et al., 1997; Bavis, 2005; Bavis et al., 2010) but resembles the effect of brief (10–15 min) hyperoxia in adult humans (Honda et al., 1996) and rats (Gozal, 1998; Pokorski et al., 2005). These findings confirm that prevailing O₂ levels, whether of short or long duration, can profoundly influence the respiratory control system. The diversity of this plasticity, however, emphasizes that the respiratory phenotype may reflect simultaneous changes in multiple cellular and/or anatomical pathways. Although the present data did not reveal a role for hyperoxia-induced production of O₂^{•-} in this plasticity, future

studies should consider the potential involvement of other reactive oxygen and/or nitrogen species.

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Fig. 1.

(A) Baseline (21% O_2) minute ventilation, (B) tidal volume, and (C) respiratory frequency for neonatal rats exposed to 21% O_2 (Control) or 60% O_2 (Hyperoxia) for the preceding 23–28 hours. Individuals were injected with saline ($n=15$ Control, 12 Hyperoxia) or the antioxidant drug MnTMPyP ($5\text{--}10 \text{ mg kg}^{-1}$, i.p.) ($n=11$ Control, 10 Hyperoxia). Values are mean $\pm$ SEM. Symbols denote significant ($P < 0.05$) main effects for hyperoxia treatment (*) or drug treatment (†) based on a 2-way ANOVA.

Fig. 2.

Ventilatory responses to 12% O₂ for neonatal rats exposed to 21% O₂ (Control) or 60% O₂ (Hyperoxia) for the preceding 23–28 hours. Individuals were injected with saline or the antioxidant drug MnTMPyP (5–10 mg kg⁻¹, i.p.). Increases in (A) minute ventilation, (B) tidal volume, and (C) respiratory frequency are presented as a percentage of baseline during the early (minute 1) or late (minute 8) phases of the response. Values are mean±SEM. Sample sizes and symbols for statistical comparisons are the same as in Fig. 1.

Fig. 3. (A) Baseline (21% O₂) minute ventilation and the (B) early (minutes 1 and 2) and (C) late (minute 8) ventilatory responses to 12% O₂ for neonatal rats exposed to 21% O₂ (Control, n=12) or 60% O₂ (Hyperoxia, n=13) for the preceding 23–28 hours. All rats were administered 5–10 mg kg⁻¹ (i.p.) MnTMPyP twice, once at the start of the exposure and once 12 h into the exposure (i.e., MnTMPyP ×2). Values are mean±SEM. * denotes significantly different groups ($P<0.05$).

A) Baseline ventilation **B) Early HVR** **C) Late HVR** **Fig. 4.**

(A) Baseline (21% O₂) minute ventilation and the (B) early (minutes 1 and 2) and (C) late (minute 8) ventilatory responses to 12% O₂ for neonatal rats exposed to 21% O₂ (Control, n=16) or 60% O₂ (Hyperoxia, n=18) for the preceding 30 min. Values are mean±SEM. No significant differences were detected between groups.