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Perinatal hyperoxia exposure impairs hypoxia-induced depolarization in rat carotid body glomus cells

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ABSTRACT

Chronic post-natal hyperoxia reduces the hypoxic ventilatory response by reducing the carotid body sensitivity to acute hypoxia as demonstrated by a reduced afferent nerve response, reduced calcium response of carotid body glomus cells and reduced catecholamine secretion in response to acute hypoxia. The present study examined whether hyperoxia alters the electrophysiological characteristics of glomus cells. Rats were treated with hyperoxia for 1 week starting at P1 or P7 and for 2 weeks starting at P1 followed by harvesting and dissociation of their carotid bodies for whole cell, perforated-patch recording. As compared to glomus cells from normoxia animals, hyperoxia treated cells showed a significant reduction in the magnitude of depolarization in response to hypoxia and anoxia, despite little change in the depolarizing response to 20 mM K^+. Resting cell membrane potential in glomus cells from rats exposed to hyperoxia from P1 to P15 and studied at P15 was slightly depolarized compared to other treatment groups and normoxia-treated cells, but conductance normalized to cell size was not different among groups. We conclude that postnatal hyperoxia impairs carotid chemoreceptor hypoxia transduction at a step between hypoxia sensing and membrane depolarization. This occurs without a major change in baseline electrophysiological characteristics, suggesting altered signaling or alterations in the relative abundance of different leak channel isoforms.

1. Introduction

The acute hypoxic ventilatory response (HVR) is markedly attenuated in adult animals exposed to perinatal hyperoxia in the newborn period (Bavis et al., 2011a). This plasticity only occurs if the hyperoxia exposure is during the first month of life in rats, a 'critical period' of carotid body development (Carroll, 2003). In contrast, equivalent chronic hyperoxia exposure of adult animals does not produce lasting respiratory impairment (Ling et al., 1996). The issue is of some clinical importance because of the prevalent use of oxygen supplementation in the clinical setting, for instance, in the treatment of premature infants.

This attenuation of the HVR is due to carotid chemoreceptor dysfunction as evidenced by an impaired whole carotid sinus nerve response to moderate hypoxia, asphyxia and cyanide which are, at least in part, due to axonal loss (Bisgard et al., 2003; Erickson et al., 1998) and a reduced number of oxygen-sensing cells (glomus cells) within the carotid body (CB) (Dmitrieff et al., 2012). Besides axonal loss of chemoreceptor afferents, perinatal hyperoxia impairs the spiking response of the remaining chemoreceptor fibers, a reversible impairment that lasts less than 7–8 days following a return to normoxia (Bavis et al., 2011b).

The impairment of organ function in the immediate post-hyperoxic period appears to be due to an alteration in the biophysical response of the glomus cell to acute hypoxia. Although acute hypoxia generally raises intracellular calcium ($[Ca^{2+}]_i$) levels in glomus cells, the response is severely blunted by hyperoxia exposure. For instance, hyperoxia exposure of neonatal rats from P7 to P12 blunts the $[Ca^{2+}]_i$ response to acute hypoxia challenge and nearly abolishes the response when hyperoxia is continued to P14 (Donnelly et al., 2009). As described for single-unit activity, this profound impairment of glomus cell $[Ca^{2+}]_i$ responses to acute hypoxia can be fully reversed after 7–8 days of recovery in normoxia (Bavis et al., 2011b). As expected for the blunted calcium response, hypoxia-induced vesicle release from glomus cells is similarly reduced by hyperoxia exposure (Donnelly et al., 2009).

The mechanism of hyperoxia-induced blunting of the glomus cell $[Ca^{2+}]_i$ response to acute hypoxia is unknown. It is

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well-established that the rise in $[Ca^{2+}]_i$ with acute hypoxia is primarily due to depolarization and calcium influx via voltage-gated calcium channels; intracellular calcium release appears to play only a minor role (Buckler and Vaughan-Jones, 1994). Hyperoxia could potentially impair this process by several mechanisms. For instance, hyperoxia may ablate the oxygen-sensing signal or induce expression of a channel that stabilizes membrane potential at hyperpolarized levels. The present study was undertaken to determine whether perinatal hyperoxia exposure alters the resting electrophysiological characteristics of CB glomus cells and/or leads to blunting of cell membrane depolarization in response to acute hypoxia. Some of these experiments were presented at the meeting of the International Society for Arterial Chemoreception (ISAC) in 2011 (Kim et al., 2012).

2. Methods

2.1. Ethical approval

The use of animals in this study was approved by the Animal Care and Use Committee of the University of Arkansas for Medical Sciences.

2.2. Animal model

Experiments were conducted on Sprague-Dawley rat pups of both sexes. Starting on postnatal day 1 (P1) or postnatal day 7 (P7) rats were placed in an environmental chamber (OxyCycler, BioSpherix) for one week or two weeks. For exposure starting at P1, pregnant rats were placed in the chamber 1–2 days prior to expected delivery and were allowed to give birth. The chamber atmosphere was maintained at 60% O_2 and chamber CO_2 was maintained under 0.2% by a controlled leak; both variables were continuously monitored (AnaWin2 Run-Time, ver. 2.4.17, Watlow-Anafaze). Animals remained in the chamber until studied at P8 or P15. Control animals maintained in normoxia were housed in the same room and carotid bodies were harvested at the same ages.

Three experimental groups were employed. The first utilized hyperoxia exposure from P1 to P8, which we have previously demonstrated causes a severe impairment of the afferent nerve response to hyperoxia (Donnelly et al., 2009). Comparison is made to a control group raised in normoxia from P1 to P8. The second group was exposed to hyperoxia from P1 to P15 and compared to a control group raised in normoxia from P1 to P15 to determine whether a longer treatment period produced greater effects. In the third group, rats were raised in normoxia from P1 to P7, exposed to hyperoxia from P7 to P15 and compared to control rats raised in normoxia from P1 to P15. This initial exposure to normoxia from P1 to P7 allowed for the normal postnatal maturation of chemoreceptor function to take place prior to hyperoxia exposure. These three groups are referred to as Hyper 1–8, Hyper 7–15 and Hyper 1–15, respectively.

2.3. Carotid body cell isolation and experimental groups

Carotid bodies were harvested as previously described (Wasicko et al., 2006). Rat pups were anesthetized with isoflurane, decapitated, and the heads placed in ice-cold buffered saline solution (BSS: 118 mM NaCl, 23 mM $NaHCO_3$, 3 mM KCl, 2 mM KH_2PO_4, 1.2 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM Glucose, pH 7.2). CBs were isolated and placed in ice-cold low Ca^{2+}/Mg^{2+} phosphate buffered saline solution (PBS: 137 mM NaCl, 2.8 mM KCl, 2 mM KH_2PO_4, 0.07 mM $CaCl_2$, 0.05 mM $MgCl_2$, pH 7.4). Each CB was sectioned 2–3 times and placed in a solution containing trypsin (1 mg/ml) and collagenase (1 mg/ml) in low Ca^{2+}/Mg^{2+} PBS and incubated at 37 °C for 20–25 min. CBs were gently triturated using a fire polished Pasteur pipette to mechanically dissociate the cells. Enzymatic digestion was continued for an additional few minutes when necessary. CB growth medium (Ham's F-12, 10% fetal bovine serum, 23 mM glucose, 2 mM L-alanyl-glutamine (Glutamax-1), 10 K units penicillin/streptomycin, and 0.08 unit/ml insulin) was added to stop enzyme activity. After brief trituration, the solution containing the dissociated CBs was centrifuged for 5 min at ~2000 × g using a microcentrifuge. Supernatant was removed and warm CB growth media were added to resuspend the pellet. This step was repeated to remove traces of enzymes. A small drop of suspended CB cells was placed on glass coverslips coated with poly-D-lysine, and incubated at 37 °C for 40–45 min to allow settling and attachment of the cells. CB growth medium was further added to the cells and then incubated at 37 °C for additional 2 h. Coverslips were then transferred to the recording chamber for electrophysiological experiments. Clusters of 2–5 cells were identified and cells within this cluster that showed a granular cell surface were chosen for electrophysiological studies.

2.4. Electrophysiological studies

All voltage and current clamp recordings were performed using the perforated patch whole-cell recording technique. Experiments were conducted using an EPC9 amplifier (HEKA). Current clamp protocols were generated using Patchmaster software (HEKA). Electrodes were made from borosilicate glass capillaries (Warner Instrument Corp.). The pipette solution for all recording conditions contained (mM): 70 K_2SO_4, 30 KCl, 2 $MgCl_2$, 1 EGTA and 10 HEPES; pH was adjusted to 7.2 with NaOH. The pipette tips were filled with this solution then back-filled with the same solution containing 240–360 μg/ml amphotericin B. Pipette resistance with this solution was approximately 2–3 MΩ. The bath was grounded through an Ag–AgCl pellet with a 3 M KCl agar-bridge. Only recordings in which the access resistance <100 MΩ were accepted. Voltage measures were not corrected for the loss of the liquid junction potential between the pipette and bath solution (~7.4 mV) and represents a constant value in all recordings.

The standard bicarbonate-buffered perfusion solution contained (mM): 117 NaCl, 4.5 KCl, 23 $NaHCO_3$, 1 $MgCl_2$, 2.5 $CaCl_2$, and 12 glucose equilibrated with either 21% O_2/5% CO_2/balance N_2 or 0% O_2/5% CO_2/balance N_2 at 35 °C: pH was adjusted to 7.4 with NaOH. The same perfusion solution was used for measurement of responses to high extracellular K^+ except for equimolar substitution of 20 mM KCl for NaCl. Osmolarity was 290–300 mOsm for all solutions. The temperature of the perfusion solutions was kept at 35.0 ± 0.5 °C and the rate of perfusion was 1.8–2.2 ml/min. The partial pressure of O_2 was monitored in the perfusion line using a flow-through probe (OM-4 Oxygen Meter, Microelectrode, Inc.) and in the chamber using needle oxygen electrode (Diamond General Development Corp, Ann Arbor, MI); electrodes were calibrated using 0% O_2 solution (gassed with 95% N_2/5% CO_2/balance N_2 for 60 min followed by addition of 0.5 mM dithionite) and 21% O_2 solution (gassed with air/5% CO_2 for 60 min at 35 °C). Solutions were delivered to the recording chamber through gas-impermeable stainless steel tubing. By using a small volume (<100 μl) recording chamber, the bath exchange could be achieved in <0.5 min.

The passive membrane characteristics of membrane capacitance (C_m), membrane resistance (R_m), and access resistance (R_a) were estimated from the response to a step hyperpolarization of 20 mV from a holding potential of −80 mV. The current response was fitted to a single-order exponential. R_a was estimated from the magnitude of the current step at the start of the hyperpolarization and R_m was estimated from the steady-state current response. C_m