

Postnatal development of carotid body glomus cell response to hypoxia

M.J. Wasicko^c, G.E. Breitwieser^b, I. Kim^a, J.L. Carroll^{a,*}

^a University of Arkansas for Medical Sciences, College of Medicine, Department of Pediatrics, Little Rock, AR, United States

^b Weis Center for Research, Geisinger Clinic, Danville, PA, United States

^c Johns Hopkins University School of Medicine, Johns Hopkins Children's Center, Baltimore, MD, United States

Accepted 11 January 2006

Abstract

This study examines developmental changes in CB glomus cell depolarization, intracellular calcium ($[Ca^{2+}]_i$) and the magnitude of an O_2 -sensitive background ionic conductance that may play roles in the postnatal increase in oxygen sensitivity of glomus cells isolated from rats of 1–3 days and 11–14 days postnatal age. Using fura-2 and perforated patch whole cell recordings, we simultaneously measured $[Ca^{2+}]_i$ and membrane potential (E_m) during normoxia and hypoxia. Resting E_m in normoxia was similar at both ages. Hypoxia caused a larger E_m depolarization and correspondingly larger $[Ca^{2+}]_i$ response in glomus cells from 11- to 14-day-old rats compared to 1–3-day-old rats. E_m and $[Ca^{2+}]_i$ responses to 40 mM K^+ were identical between the two age groups. Under normoxic conditions both age groups had similar background conductances. Under anoxic conditions (at resting membrane potential) background K^+ conductance decreased significantly more in cells from 11- to 14-day-old rats compared to cells from 1- to 3-day-old rats. Glomus cells from newborns therefore have less O_2 -sensitive background K^+ conductance. These results support the hypothesis that postnatal maturation of glomus cell O_2 sensitivity involves developmental regulation of the expression and/or O_2 -sensitivity of background ionic conductances.

© 2006 Elsevier B.V. All rights reserved.

Keywords: Potassium channel; Carotid body; Hypoxia; Development; Chemoreceptor

1. Introduction

The carotid body (CB) chemoreceptors are the main sensors of arterial oxygen level in mammals (Gonzalez

et al., 1994). Their inputs to brainstem respiratory control nuclei drive the ventilatory response to hypoxia and other defensive responses to hypoxic stress (Hofer, 1984, 1986; Bureau et al., 1985a,b; Fewell et al., 1989a,b, 1990). In neonates but not adults, carotid denervation leads to high mortality rates and abnormalities of respiratory control (Hofer, 1984, 1986; Bureau et al., 1985a; Donnelly and Haddad, 1990; Cote et al., 1996), suggesting a vulnerable period during mammalian postnatal development during which the CB is

* Corresponding author at: Pediatric Pulmonary Section, Slot 512-17, Arkansas Children's Hospital, 800 Marshall Street, Little Rock, AR 72202, United States. Tel.: +1 501 364 1006; fax: +1 501 364 3930.

E-mail address: carrolljohnl@uams.edu (J.L. Carroll).

important for survival and normal maturation of breathing control. Despite their importance in the developing infant, the carotid chemoreceptors have low sensitivity to hypoxia at birth and become more sensitive over the first few days or weeks of life (Biscoe and Purves, 1967; Blanco et al., 1984; Mulligan and Lahiri, 1987; Marchal et al., 1992; Kholwadwala and Donnelly, 1992; Carroll et al., 1993); a process termed “resetting”. The mechanism(s) underlying resetting are unknown.

According to the most widely accepted model of CB oxygen transduction, the oxygen sensing elements of the CB are the glomus cells. Exposure to hypoxia leads to glomus cell depolarization, Ca^{2+} influx through voltage-gated calcium channels, increased intracellular calcium ($[\text{Ca}^{2+}]_i$) and secretion of neurotransmitter(s), which increase the firing rate of action potentials in the adjacent carotid sinus nerve (CSN) terminals. Although CB resetting may involve changes in neurotransmitter secretion, receptor expression or other synaptic mechanisms, the purpose the present study was to explore developmental changes in the glomus cell depolarization response to hypoxia. We previously reported that, in rat glomus cells, $[\text{Ca}^{2+}]_i$ responses to graded hypoxia increase with postnatal age, following the same time course as maturation of the CSN response to hypoxia (Bamford et al., 1999). Most of the increase in rat glomus cell O_2 sensitivity occurs between 3 and 14 days of age, with apparent maturity reached in this species by 14–21 days (Wasicko et al., 1999). Whether the magnitude of glomus cell depolarization also increases with age is unknown.

The mechanisms underlying maturation of glomus cell O_2 sensitivity are unknown. As cell membrane depolarization appears to be a critical step in the O_2 chemosensory cascade, we hypothesized that the postnatal increase in the glomus cell response to hypoxia may reflect age-related changes in the magnitude of hypoxia-induced depolarization. Glomus cell depolarization by hypoxia results from inhibition of K^+ currents active at resting membrane potential, most likely non-voltage sensitive, background (“leak”) K^+ channels (Buckler, 1997) and possibly large-conductance, Ca^{2+} -activated K^+ channels (K_{Ca}) (Peers, 1990). Activity and O_2 sensitivity of rat glomus cell K_{Ca} channels increases between 4 and 10 days of age, consistent with a possible role in postnatal maturation of glomus cell O_2 sensing (Hatton et al., 1997). However, postnatal development of glomus cell O_2 -sensitive back-

ground K^+ currents has not been characterized. The specific aims of the present study were to determine whether developmental changes occur in the magnitude of hypoxia-induced depolarization and the inhibition of glomus cell background current by hypoxia. The results indicate that glomus cell hypoxia-induced depolarization and the O_2 -sensitive background K^+ conductance increase with age and may play a role in postnatal maturation of glomus cell O_2 sensitivity.

2. Methods

2.1. Isolation of cells

Glomus cells were isolated from rats from two age groups: 1–3 and 11–14 days old. All procedures were approved by the institutional animal care and use committee. Each rat was anesthetized with methoxyflurane, decapitated, and the head placed in ice-cold saline. The carotid bifurcations were dissected and placed in ice-cold phosphate buffered saline (PBS, Sigma). The carotid bodies were dissected from the bifurcations, cut in half and placed in 1 ml of enzymatic solution comprised of 0.6 ml of PBS with $50 \mu\text{M}$ Ca^{2+} , 0.2 ml of trypsin (1 mg ml^{-1} , Sigma), and 0.2 ml of type I collagenase (5 mg ml^{-1} , Sigma). For rats aged 1–3 days old, the carotid bodies were incubated in the enzymatic solution at 37°C in 21% $\text{O}_2/5\%$ CO_2 for 20 min, as done in our previous study (Wasicko et al., 1999) and for rats aged 11–14 days old, incubation time was 26 min. The increased incubation time for the older group was performed to better allow $\text{G}\Omega$ seals to be made using patch-clamp techniques.

The carotid bodies, along with the enzyme solution, were transferred to an Eppendorf tube with a fire polished glass pipet and incubated further; incubation time for rats aged 1–3 days old was 5 min and for rats aged 11–14 days old was 7 min. After the second incubation, the cells were dispersed by gentle trituration with a fire polished glass pipet. The tissue was pelleted at $2000 \times g$ for 2 min and resuspended in 1 ml of growth media composed of Ham’s F12 (Mediatech) with 10% fetal calf serum, 33 mM glucose, 2 mM L-glutamine, 100 units ml^{-1} penicillin, 100 $\mu\text{g ml}^{-1}$ streptomycin, and 0.08 U ml^{-1} insulin. The cells were centrifuged a second time at $2000 \times g$ for 2 min, the supernatant

removed, and growth media added at 25 μ l per coverslip. The cells were plated on poly-D-lysine coated glass coverslips and incubated at 37 °C in 21% O₂/5% CO₂ until use. Clusters of glomus cells were studied between 3 and 8 h after plating. At least three cell preparations, from different litters, were studied at each age.

2.2. Measurement of intracellular calcium

Cytosolic Ca²⁺ ([Ca²⁺]_i) was measured by quantitative fluorescence imaging using the calcium-sensitive dye fura-2 (Grynkiewicz et al., 1985). Cells attached to the coverslip were loaded with fura-2 by incubation for 8 min at 37 °C in 21% O₂/5% CO₂ with 4 mM of the fura-2 acetoxymethyl ester (fura-2 AM; Molecular Probes). Fura-2 fluorescence emission was measured at 510 nm in response to alternating excitation at 340 and 380 nm. Images were acquired and stored using a Zeiss Axiovert-135TV microscope and CCD camera with image intensifier (Videoscope) under computer control (Metafluor, Universal Imaging).

For each coverslip, the background emission at 510 nm was determined and subtracted, pixel-by-pixel, from each image before measurement of the fluorescence intensity ratio at 340 nm/380 nm. [Ca²⁺]_i was determined using the 340/380 fluorescence ratio and the following equation:

$$[\text{Ca}^{2+}]_i = K_d \left(\frac{R_0 - R_{\min}}{R_{\max} - R_0} \right) \beta$$

where R_0 is the measured fluorescence ratio, R_{\min} the fluorescence ratio at 0 Ca²⁺, R_{\max} the fluorescence ratio at saturating Ca²⁺, K_d the dissociation constant for fura-2, 224 nM (Grynkiewicz et al., 1985), and β is the ratio of 380 nm fluorescence intensity at 0 Ca²⁺ to 380 nm fluorescence intensity at saturating Ca²⁺ concentrations. Calibration was performed using cell-free solutions. Autofluorescence was determined by treating the cells as above but without loading with fura-2. At the maximum camera gains used for this preparation, autofluorescence was negligible under control or anoxic conditions.

2.3. Electrophysiology

All voltage and current clamp recordings were performed using the perforated patch whole-cell recording technique. Experiments were conducted using an

Axopatch 200B (Axon Instruments). Voltage pulse protocols were generated using pClamp 7.0 software (Axon Instruments). Electrodes were made from borosilicate glass capillaries (Warner Instrument Corp.). The pipet solution for all recording conditions contained (in mM): 140 potassium gluconate, 5 MgCl₂, 1 EGTA and 10 HEPES; pH was adjusted to 7.2 at 37 °C with NaOH. The pipet tips were filled with this solution then back-filled with the same solution containing 240–360 μ g/ml amphotericin B. Pipet resistance with this solution was approximately 3–5 M Ω . The bath was grounded through an Ag–AgCl pellet with a 3 M KCl bridge. Liquid junction potentials were offset prior to seal formation.

The standard superfusate contained (in mM): 117 NaCl, 3.5 KCl, 23 NaHCO₃, 1 MgCl₂, 2.5 CaCl₂, and 12 glucose equilibrated with either 21% O₂/5% CO₂ or 0% O₂/5% CO₂ at 37 °C (superfusate 1). The same superfusate was used for measurement of responses to high extracellular K⁺ except for equimolar substitution of either 20 or 40 mM KCl for NaCl. The superfusate used when recording the O₂ sensitivity of the background K⁺ currents (superfusate 2) contained (in mM): 100 NaCl, 3.5 KCl, 23 NaHCO₃, 3.5 MgCl₂, 10 tetraethylammonium (TEA), 5 4-aminopyridine (4-AP), and 12 glucose, equilibrated with 21% O₂/5% CO₂ at 37 °C. TEA/4-AP, which blocks Ca²⁺ activated, voltage gated, ATP sensitive and KCNQ K⁺ channels, but not two-pore K⁺ channels, was included in superfusate 2 in order to improve isolation of O₂-sensitive background K⁺ currents (Buckler, 1997). Osmolarity was 290–300 mOsm for all solutions. The difference in osmolality between each of the perfusates and the pipette solution was less than 10 mOsm and solution pH was \sim 7.4. Solutions were delivered to the recording chamber with gas-impermeable tubing.

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 was estimated by dividing the time constant of the exponential by R_a .

2.4. Experimental protocols

The coverslip was placed in an open microscope chamber with a conical collar that allowed argon to flow over the surface of the superfusate to maintain PO₂ levels (protocols 1–3). PO₂ was not measured for each experiment but rather under similar experimental conditions. Only one experimental protocol was performed per coverslip.

Glomus cells have a high catecholamine content, and in our previous study (Sterni et al., 1995) characteristic glomus cell morphology (~10–15 μm diameter, rounded shape, tendency to occur in clusters) correlated well with the presence of catecholamines using glyoxylic acid-induced amine fluorescence (Nurse, 1990). In the present study, glomus cells were identified on the basis of their characteristic morphology and their occurrence in clusters. Only cells in clusters were studied. Cluster sizes ranged from 2 to 10 cells. The criteria for a successful experiment were (a) [Ca²⁺]_i should not increase by more than 50 nM as a result of formation of the perforated patch and (b) E_m should be equal to or more negative than –40 mV.

We only studied cells that were in clusters because the electrophysiological characteristics of cells in clusters may be different from single cells (Pang and Eyzaguirre, 1992), and, as indicated above, glomus cells occur in clusters. Although in preliminary studies we found [Ca²⁺]_i responses to hypoxia were similar in single cells and cells in clusters, we chose to study only clustered cells rather than mix two populations of cells that may have different electrical properties. We were initially concerned about cell-to-cell coupling within the cluster. However, we found that the charging transient in response to a hyperpolarizing voltage step was well fitted by a single exponential function, suggesting minimal cell-to-cell coupling. In addition, membrane capacitance (an indicator of membrane surface area) was approximately 6 pF, which is similar for single cells, again suggesting minimal cell-to-cell coupling.

2.4.1. Protocol 1: Simultaneous measurement of [Ca²⁺]_i and depolarization

This protocol simultaneously measured [Ca²⁺]_i and membrane potential (E_m) during exposure to hypoxia or elevated extracellular K⁺ in glomus cells from the two age groups. The rationale was to determine whether

developmental differences exist in the magnitude of depolarization and/or the relationship between depolarization and the [Ca²⁺]_i rise in response to hypoxic versus non-hypoxic depolarizing stimuli. After loading with fura-2 the cells were perfused with superfusate 1 and held at –80 mV during the formation of the perforated patch. After establishing whole-cell configuration (15–25 min after the initial seal), E_m, under current clamp conditions (I=0), and [Ca²⁺]_i were measured simultaneously. After 1–2 min of recording under normoxic conditions, the response to hypoxia was examined by superfusing cells with solution 1 equilibrated with 0% O₂/5% CO₂ for 90 s. Following 2–3 min of recovery, the response to high extracellular K⁺ was measured during a 1 min superfusion with solution 1 containing 40 mM K⁺. The Metafluor imaging software (Universal Imaging, Inc.) allows fluorescence imaging of an unequivocally delineated region of interest over the single cell being current clamped.

2.4.2. Protocol 2: O₂-sensitive background K⁺ current

Background K⁺ conductance in glomus cells from the two age groups was measured using techniques similar to those described by Buckler (Buckler and Vaughan-Jones, 1994; Buckler, 1999). The rationale was to detect developmental differences in (1) membrane conductance at resting membrane potential, (2) inhibition of background conductance by hypoxia and (3) inhibition of background conductance by Ba²⁺, a blocker of background K⁺ conductance. Cells were perfused with solution 1, at a holding potential of –80 mV, until the formation of a perforated patch. After stabilization of the perforated patch condition (no further decreases in resistance), control background K⁺ current was measured by applying a 500 ms voltage ramp from –100 to –40 mV every 5 s. The O₂-sensitive background K⁺ current was measured by superfusing the cells with solution 1 equilibrated with 0% O₂/5% CO₂ + 0.5 mM sodium hydrosulfite (Na₂S₂O₄) (PO₂ ~ 0 mmHg) for 1 min. The ramp protocol was then repeated. After 2 min of recovery from the anoxic challenge, the Ba²⁺-sensitive background K⁺ current was blocked by perfusing the cells with solution 1 equilibrated with 21% O₂/5% CO₂ + 5 mM BaCl₂ for 1 min. Currents were again assessed with the ramp protocol.

2.4.3. Protocol 3: Background K^+ current response to graded hypoxia

This protocol measured the response of background K^+ conductance to graded hypoxia in order to detect developmental differences in the magnitude of hypoxia-induced inhibition of background K^+ conductance. The cells were perfused with solution 1 until the formation of a perforated patch. Holding potential was -80 mV. The perfusate was then switched to solution 2 and background K^+ currents were obtained from 300 ms depolarizing pulses ranging from -60 to -20 mV, from a holding potential of -80 mV. The effect of graded hypoxia on background K^+ currents was examined by superfusing the cells with solution 2 equilibrated with hypoxic gas mixtures containing 1% O_2 , 0% O_2 , and 0% O_2 + 0.5 mM sodium hydro-sulfite ($Na_2S_2O_4$) (all with 5% CO_2). The PO_2 of the superfusate was measured at the inlet of the recording chamber using a flow-through oxygen electrode (Microelectrodes, Inc.). For each hypoxic condition, the cells were perfused for 1 min with the hypoxic superfusate and currents recorded using the above protocol. In each cell studied, currents were recorded during normoxia, all three hypoxic conditions, and recovery.

2.4.4. Protocol 4: Effect of TEA/4-AP on $[Ca^{2+}]_i$ response to hypoxia

Using fura-2, this protocol measured the $[Ca^{2+}]_i$ response to hypoxia in the absence and presence of 10 mM TEA and 5 mM 4-AP, in glomus cells from 1- and 10–11-day-old rats. Glomus cells were superfused in a closed imaging chamber (Warner Instruments) at $37^\circ C$ with bicarbonate-buffered salt solution (BSS) (superfusate 1) equilibrated with 21% O_2 /5% CO_2 at $37^\circ C$. The $[Ca^{2+}]_i$ response to hypoxia was tested three consecutive times, separated by at least 5 min, by switching the superfusate to BSS equilibrated with 0% O_2 /5% CO_2 for 2 min. During the second hypoxia challenge, the superfusate also contained 10 mM TEA and 5 mM 4-AP (pH adjusted to ~ 7.4). The effect of 10 mM TEA + 5 mM 4-AP on glomus cell $[Ca^{2+}]_i$ was also tested during normoxia.

2.5. Data analysis

For fluorescence data, baseline values were calculated as the average $[Ca^{2+}]_i$ during the 2 min period

prior to a challenge and peak values of $[Ca^{2+}]_i$ were the maximum obtained during a 90 s challenge. $\Delta[Ca^{2+}]_i$ was calculated as peak $[Ca^{2+}]_i$ – baseline $[Ca^{2+}]_i$.

E_m was measured as the mean voltage over a 5 s period for each experimental condition. For analysis of background K^+ conductance, current–voltage relationships were determined by averaging the current obtained from five consecutive voltage ramps. The O_2 -sensitive conductance was obtained by subtracting, at each voltage, the current recorded during anoxia from that recorded during normoxia. The Ba^{2+} -sensitive conductance was obtained by subtracting, at each voltage, the current recorded during perfusion with 5 mM $BaCl_2$ from that recorded during normoxia. For analysis of the O_2 sensitivity (graded hypoxia) of background K^+ currents, the current obtained during the last 50 ms of a voltage step was expressed as a function of PO_2 of the perfusate.

Values are presented as mean \pm S.E.M. Comparisons of passive membrane characteristics and change in E_m and $[Ca^{2+}]_i$ (Figs. 2A and B and 3A and B) between the two age groups were performed using two sample *t*-tests assuming unequal variances. E_m values at two ages and at two levels of PO_2 were tested using two-way ANOVA with Bonferroni post hoc testing. For the graded hypoxia protocol (Fig. 5), current at different PO_2 values and age groups were compared using two-way analysis of variance for repeated measures, with Bonferroni post hoc testing to compare between age groups for a given level of PO_2 (Graphpad Prism 4.03). The effects of age and TEA/4-AP on $[Ca^{2+}]_i$ responses to hypoxia (Fig. 6) were tested by two-way analysis of variance for repeated measures (SigmaStat for Windows Version 3.11) using two approaches. The first approach used two-way ANOVA for repeated measures with age as the between-subjects factor (two levels) and oxygen/drug condition as the within-subjects factor (eight levels, e.g., (1) baseline, (2) first hypoxia challenge, (3) second baseline measurement, etc.). The second approach, in order to correct for possible effects of differing baseline $[Ca^{2+}]_i$ values, used two-way ANOVA for repeated measures to compare $\Delta[Ca^{2+}]_i$ responses (peak – baseline), with age as the between-subjects factor (two levels) and $\Delta[Ca^{2+}]_i$ responses to each challenge as the within-subjects factor (four levels, e.g., (1) first hypoxia challenge, (2) TEA/4-AP in normoxia, etc.). For both approaches, Bonferroni post

hoc testing was employed. A p -value < 0.05 was considered statistically significant.

3. Results

3.1. Postnatal development of passive membrane characteristics

The passive membrane characteristics of capacitance (C_m) and membrane resistance (R_m) of the glomus cells from both age groups were measured from the current response to a 20 mV hyperpolarizing pulse from a holding potential of -80 mV. In cells from 1- to 3-day-old rats ($n = 17$), $C_m = 5.9 \pm 0.6$ pF and $R_m = 1.8 \pm 0.2$ G Ω , and in cells from 11- to 14-day-old rats ($n = 18$), $C_m = 5.3 \pm 0.4$ pF and $R_m = 2.0 \pm 0.2$ G Ω . Neither C_m nor R_m was statistically different between the two age groups.

3.2. Postnatal development of $[Ca^{2+}]_i$ and E_m responses to hypoxia

Hypoxia is known to cause depolarization and increased $[Ca^{2+}]_i$ in glomus cells. Our results show that both of these responses change with age. Fig. 1 shows representative simultaneous recordings of $[Ca^{2+}]_i$ and E_m during exposure to hypoxia or 40 mM extracellular K^+ in cells from 3- and 11-day-old rats. In a cell

from 3-day-old rats (left panel), the magnitude of the $[Ca^{2+}]_i$ response to hypoxia was smaller compared to that in a cell from 11-day-old rats (right panel) and was associated with a correspondingly smaller depolarization. In contrast $[Ca^{2+}]_i$ responses to 40 mM K^+ were similar at both ages, as previously reported (Wasicko et al., 1999), and the corresponding membrane potential responses to 40 mM K^+ also did not differ with age.

Fig. 2 shows the mean $[Ca^{2+}]_i$ and E_m responses to hypoxia from simultaneous recordings. Consistent with our previous findings, the $\Delta[Ca^{2+}]_i$ response to hypoxia was significantly larger in glomus cells from older rats compared to cells from newborns (Fig. 2B). Resting membrane potential in normoxia was ~ 51 – 53 mV and did not vary with age (Fig. 2A). Significant interaction between age and PO_2 ($p < 0.05$) was revealed by two-way ANOVA; hypoxia induced a larger magnitude depolarization in cells from 11- to 14-day-old rats versus 1- to 3-day-old rats (Fig. 2A) ($p < 0.001$, Bonferroni post hoc test). The average change in E_m (ΔE_m) in cells from 1- to 3-day-old rats was ~ 8 mV which was significantly smaller than the average ΔE_m of ~ 18 mV in cells from 11- to 14-day-old rats. Age-related differences in the magnitude of depolarization corresponded with similar age-related differences in $[Ca^{2+}]_i$ (Fig. 2B).

The average responses of membrane potential and $[Ca^{2+}]_i$ to challenge with 40 mM extracellular K^+ are shown in Fig. 3. Elevated extracellular K^+

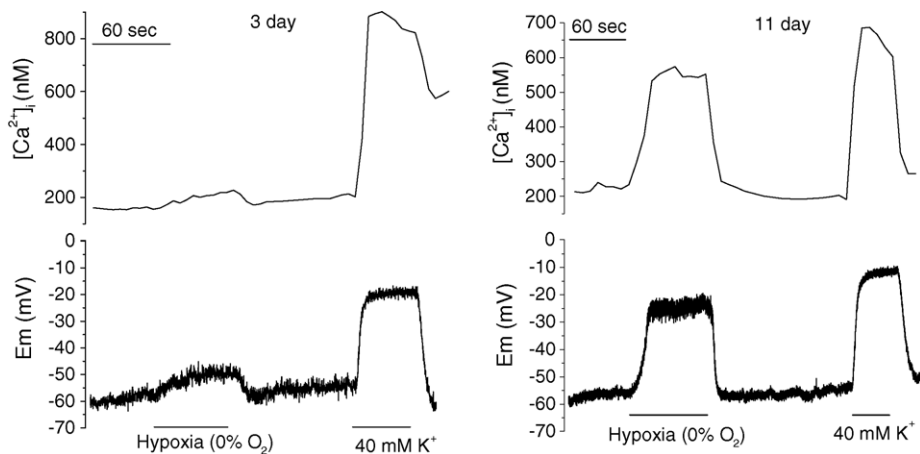


Fig. 1. Representative $[Ca^{2+}]_i$ and E_m responses to hypoxia and 40 mM K^+ . Traces show representative simultaneous $[Ca^{2+}]_i$ and E_m recordings from single glomus cells of 3-day-old (left panel) and 11-day-old rats (right panel). Recordings made in current clamp ($I = 0$) mode.

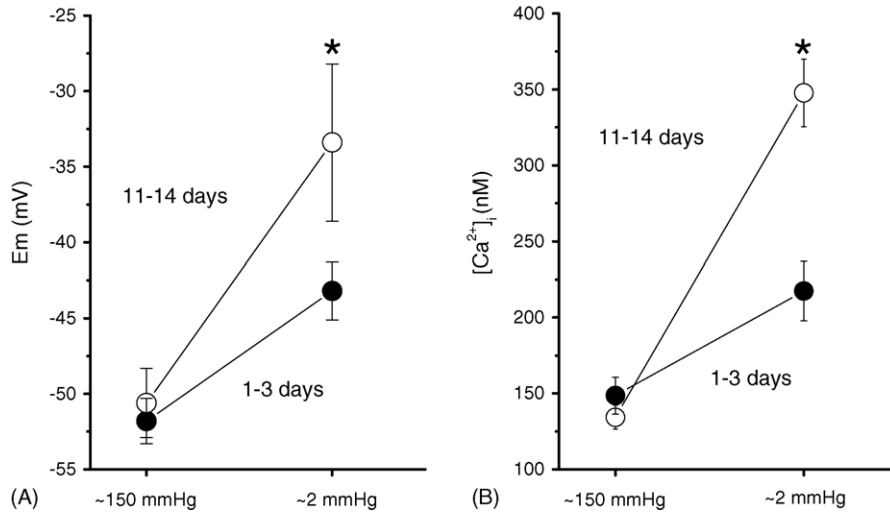


Fig. 2. Mean E_m , $[Ca^{2+}]_i$ responses to hypoxia. Mean (A) E_m and (B) $[Ca^{2+}]_i$ responses recorded simultaneously in cells from 1- to 3-day-old ($n=20$) and 11- to 14-day-old ($n=25$) rats in normoxia ($PO_2 \sim 150$ mmHg) and in response to hypoxia ($PO_2 \sim 2$ mmHg). Filled symbol = 1–3 days, open symbol = 11–14 days. Values are mean \pm S.E.M. *Statistical difference ($p < 0.001$) between the two age groups.

caused a much larger depolarization than hypoxia, which was accompanied by correspondingly larger $\Delta[Ca^{2+}]_i$ responses (Fig. 3B). Contrasting sharply with responses to hypoxia (Fig. 2), E_m , and $[Ca^{2+}]_i$ responses to 40 mM K^+ were not different between the two age groups (Fig. 3).

3.3. Postnatal development of background K^+ conductance

In order to examine the background currents active around the resting membrane potential (protocol 2 in methods), current–voltage relationships

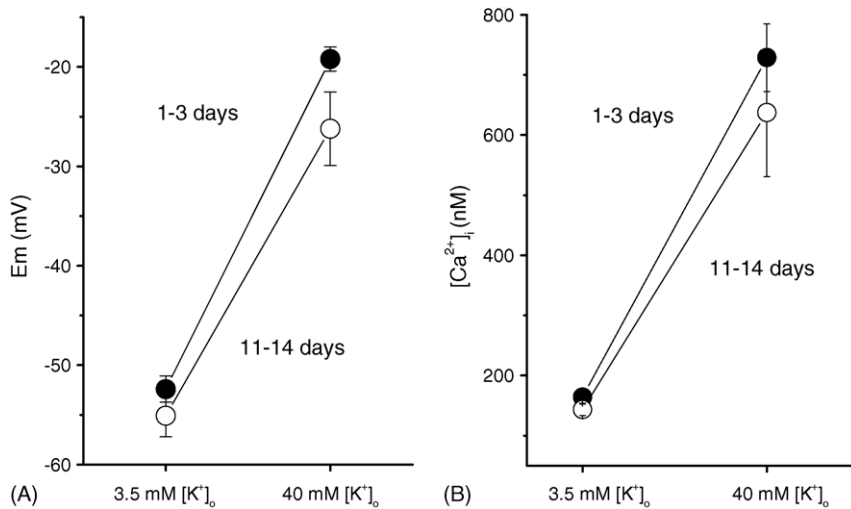


Fig. 3. Mean E_m , ΔE_m , and $\Delta[Ca^{2+}]_i$ responses to 40 mM K^+ . Mean (A) E_m and (B) $[Ca^{2+}]_i$ responses recorded simultaneously in cells from 1- to 3-day-old ($n=11$) and 11- to 14-day-old ($n=14$) rats in response to 40 mM K^+ . Filled symbol = 1–3 days, open symbol = 11–14 days. Values are mean \pm S.E.M. There were no significant differences between the two age groups.

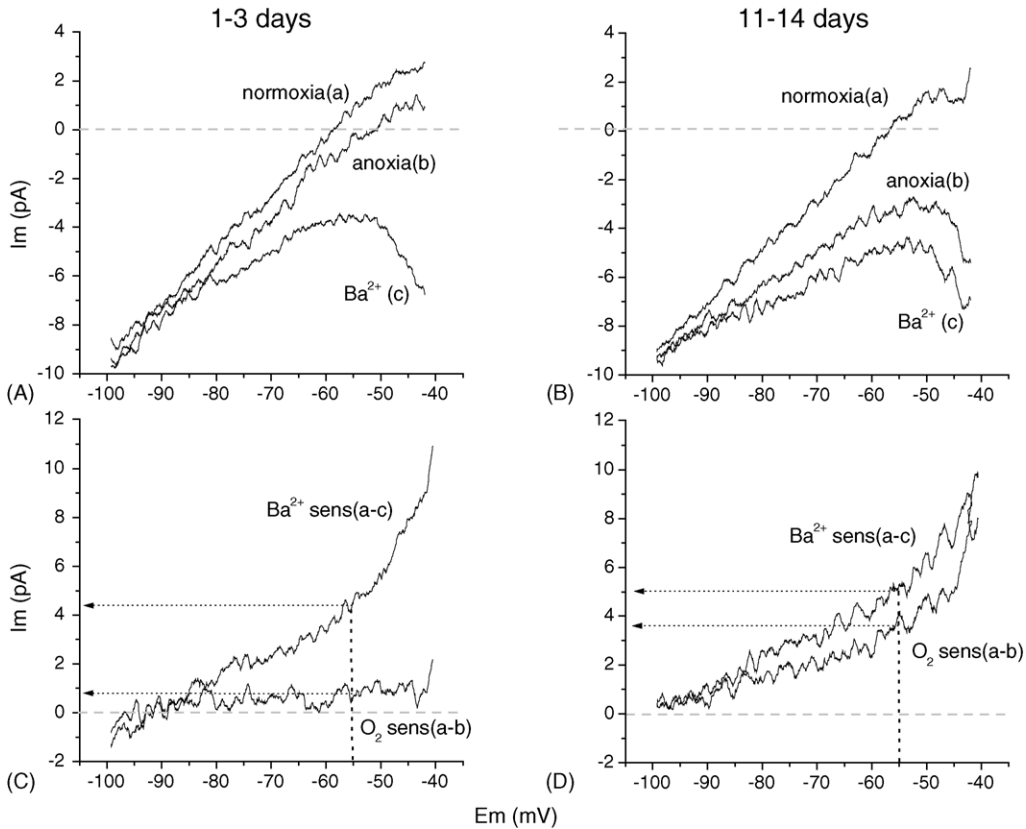


Fig. 4. Postnatal development of O₂-sensitive background current. From a holding potential of -80 mV, glomus cells were subjected to 500 ms voltage ramps from -100 to -40 mV. (A) 1–3 days old ($n = 10$). Average current–voltage relationship obtained under three conditions as follows: (a) normoxia, (b) anoxia and (c) 5 mM Ba²⁺ during normoxia. (B) The same protocol as in (A) applied to cells from 11- to 14-day-old rats ($n = 15$) yielded the same I – V relationship during normoxia and exposure to 5 mM Ba²⁺ as seen in cells from 1- to 3-day-old rats. (C) 1–3 days old, I – V relationships of the O₂-sensitive current (difference current, a–b) and the Ba²⁺ sensitive current (a–c). (D) 11–14 days old, I – V relationships of the O₂-sensitive current (difference current, a–b) and the Ba²⁺ sensitive current (a–c).

were determined during periodic 500 ms voltage ramps between -100 and -40 mV, from a holding potential of -80 mV. The mean current voltage relationships obtained from voltage ramps under control and anoxic conditions as well as during exposure to 5 mM Ba²⁺ are shown in Fig. 4. In normoxia, in both age groups, the current voltage tracing crosses the zero-current axis at approximately -55 to -60 mV, which corresponds roughly to the cell's resting membrane potential under control conditions (Fig. 4A and B).

In cells from 11- to 14-day-old rats, exposure to anoxic conditions caused a marked decrease in the slope of the current–voltage relationship, resulting in a downward (inward or depolarizing) shift in the

current–voltage relationship (Fig. 4B, curve b). Five mM Ba²⁺, which effectively inhibits all K⁺ currents, also caused a marked flattening of the current–voltage relation (inward or depolarizing shift) (Fig. 4B, curve c). Thus, both 5 mM Ba²⁺ and anoxia cause a marked decrease in resting membrane conductance in cells from 11- to 14-day-old rats, as previously reported (Buckler, 1999). The difference between curves a and b in Fig. 4B yields the O₂-sensitive current (that portion of the background current that was inhibited by anoxia) which is plotted in Fig. 4D (curves a and b). Similarly, subtraction of curve c from curve a yields the Ba²⁺-sensitive current (that portion of the background current that was inhibited by 5 mM Ba²⁺),

plotted in Fig. 4D (curves a–c). The Ba^{2+} sensitive current at both ages was of approximately the same magnitude.

The O_2 -sensitive background current (curves a and b in Fig. 4C and D) at all ages exhibited a reversal potential of approximately -100 mV with little voltage dependency over the range -50 to -100 mV, consistent with a background K^+ current, as previously reported for rat glomus cells (Buckler, 1997, 1999; Williams and Buckler, 2000). In order to confirm that the O_2 -sensitive background conductance was primarily carried by K^+ , current–voltage relationships under anoxic conditions were determined at two different levels of extracellular K^+ , as previously described (Buckler, 1999). In the presence of 3.5 mM extracellular K^+ the O_2 -sensitive background current showed a reversal potential of approximately -100 mV. In the presence of 20 mM extracellular K^+ , the O_2 -sensitive current–voltage relation underwent a Nernstian shift in the reversal potential to approximately -53 mV, strongly suggesting that the O_2 -sensitive background current is carried primarily by K^+ ($n=5$).

Age-related changes in the O_2 -sensitive background K^+ current were evaluated by determining the average amplitude of the current between -60 and -50 mV in cells from 1- to 3-day-old versus 11- to 14-day-old rats ($n=10$ and 15 cells, respectively). The averaged amplitude of the O_2 -sensitive K^+ current between -60 and -50 mV was 1.0 ± 0.4 pA for the 1- to 3-day-old group (Fig. 4C, curves a and b) and 4.0 ± 1.0 pA for the 11- to 14-day-old group (Fig. 4D, curves a and b) ($p=0.02$). In sharp contrast, the magnitude of the Ba^{2+} -sensitive current between -60 and -50 mV was 5.0 ± 1.3 pA for the 1- to 3-day-old group and 5.6 ± 1.2 pA for the 11- to 14-day-old group (NS) (Fig. 4C and D). Thus, the Ba^{2+} -sensitive current was not different whereas the O_2 -sensitive K^+ current was approximately four-fold larger in cells from 11- to 14-day-old versus 1- to 3-day-old rats. Assuming that 5 mM Ba^{2+} inhibits all background K^+ current, both age groups had similar background K^+ current, but in the 1–3 days group the O_2 -sensitive component accounted for $\sim 20\%$ of background K^+ current while, in the 11–14 days age group, the O_2 -sensitive component accounted for $\sim 71\%$ of background K^+ current. Thus, in the 11–14-day-old group, a greater proportion of the background K^+ conductance was O_2 sensitive.

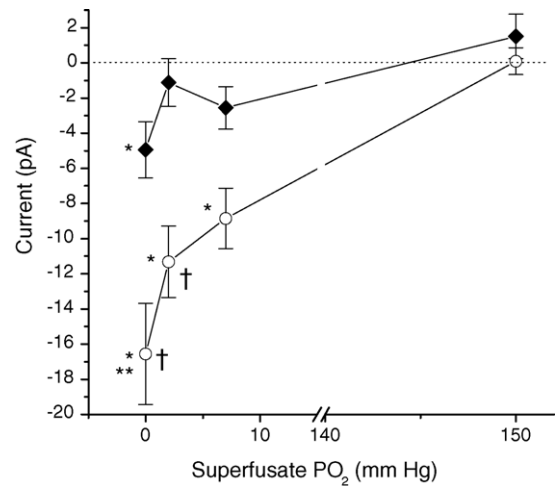


Fig. 5. Oxygen sensitivity of the background K^+ currents. O_2 sensitivity of background K^+ currents from 1- to 3-day-old rats ($n=10$) (◆) and 11- to 14-day-old rats ($n=10$) (○). All recordings made in superfusate 2. Values represent the current (mean \pm S.E.M.) at -50 mV; derived from I/V relationship generated from pulse clamp protocol (protocol #3). * $p < 0.001$, ** $p < 0.005$ different from current in normoxia within age group, † $p < 0.001$ difference between the age groups.

3.4. Effect of graded hypoxia on resting K^+ conductance

The O_2 -sensitive background K^+ conductance exhibits graded inhibition during graded hypoxia, providing a rough measure of the O_2 sensitivity of this conductance. Using protocol 3, we sought to detect age related differences in the O_2 sensitivity of glomus cell background K^+ current. In cells from 1- to 3-day-old rats, decreasing superfusate PO_2 caused a small inhibition of the background current that was only significantly decreased in anoxia (Fig. 5). In contrast, in cells from 11- to 14-day-old rats, ANOVA indicated greater inhibition of background K^+ current, compared to control, at superfusate PO_2 's of ~ 2 – 3 and 0 mmHg ($p < 0.001$). The current level in anoxia was significantly decreased compared to the level at PO_2 7 mmHg ($p < 0.001$) and 2 mmHg ($p < 0.01$). Thus, when superfusate PO_2 was ~ 2 – 3 and 0 mmHg, the inhibition of the background K^+ conductance in the older group was several fold greater than in the cells from newborns.

The effects of graded decreases in PO_2 on background ionic conductance are shown in Table 1. Chord conductance (-60 to -50 mV) under control condi-

Table 1

Mean chord conductances (pS, -50 to -60 mV) of glomus cells from 1- to 3- and 11- to 14-day-old rats

Superfusate PO ₂	3-day old (<i>n</i> = 9)	3 day % of control	11-day old (<i>n</i> = 10)	11 day % of control	3 day vs. 11 day
Control ~ 150 mmHg	410 \pm 49	100	431 \pm 53	100	(NS)
7–8 mmHg	310 \pm 42	76	342 \pm 56	78	(NS)
2–3 mmHg	371 \pm 47	90	246 \pm 30	56	<i>p</i> = 0.03
0 mmHg (with dithionite)	299 \pm 31	73	185 \pm 21	42	<i>p</i> = 0.007

Values are mean \pm S.E.M.

tions was ~ 410 – 430 pS and did not differ between the two age groups. Although anoxia appeared to cause a small decrease in background K⁺ conductance in cells from 1- to 3-day-old rats, it was not statistically significant by ANOVA. In glomus cells from 11- to 14-day-old rats, ANOVA revealed a significant decrease (from control) in background K⁺ conductance at the two lowest PO₂ levels (*p* < 0.05). Comparing between ages, at the two lowest PO₂ values, conductance was decreased to a much greater degree in cells from older rats compared to cells from 1- to 3-day-old rats (Table 1).

3.5. Effect of TEA + 4-AP on [Ca²⁺]_i response to hypoxia

Resting [Ca²⁺]_i of glomus cells from both age groups was not affected by 10 mM TEA + 5 mM 4-AP

(Fig. 6). Baseline [Ca²⁺]_i levels did not differ significantly from other baseline calcium levels within or between age groups. Peak [Ca²⁺]_i responses to hypoxia alone increased significantly with age, as expected (Fig. 6). TEA/4-AP increased the peak [Ca²⁺]_i response from 233 \pm 17 to 344 \pm 33 nM (*p* < 0.001) at 1 day and from 368 \pm 23 to 579 \pm 42 nM (*p* < 0.001) at 10–11 days (Fig. 6). TEA/4-AP resulted in a 49 \pm 9% versus 60 \pm 12% increase in the peak [Ca²⁺]_i response to hypoxia at 1 day versus 10–11 days, respectively (NS).

In order to adjust for possible effects of individual baseline variation the data presented in Fig. 6 were also analyzed as Δ [Ca²⁺]_i (difference between baseline and peak). The Δ [Ca²⁺]_i response to hypoxia increased with age (*p* < 0.001) as expected. TEA/4-AP increased the Δ [Ca²⁺]_i response from 129 \pm 14 to 232 \pm 20 nM (*p* < 0.001) at 1 day and from 230 \pm 30 to 438 \pm 38 nM

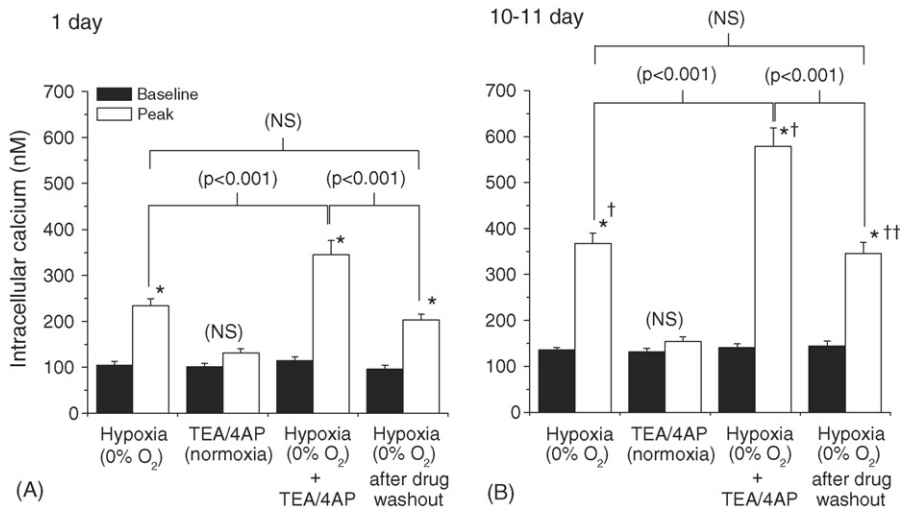


Fig. 6. Effect of TEA/4-AP on [Ca²⁺]_i response to hypoxia. Baseline (■) and peak (□) [Ca²⁺]_i responses to hypoxia in CB glomus cells from (A) 1-day-old rats (*n* = 9) and (B) 10–11-day-old rats (*n* = 11). **p* < 0.001, peak significantly different from baseline [Ca²⁺]_i. †*p* < 0.001, ††*p* < 0.005 significantly different from corresponding challenge in 1-day-old group. NS = not significantly different. None of the baseline [Ca²⁺]_i levels differed from other baseline [Ca²⁺]_i levels within or between age groups.

($p < 0.001$) at 10–11 days. TEA/4-AP resulted in an $86 \pm 24\%$ versus $96 \pm 20\%$ increase in the $\Delta[\text{Ca}^{2+}]_i$ response to hypoxia at 1 day versus 10–11 days, respectively (NS). Thus, TEA/4-AP had no effect on resting $[\text{Ca}^{2+}]_i$ and increased the $[\text{Ca}^{2+}]_i$ response to hypoxia to the same extent in both age groups.

4. Discussion

The results of the present study suggest that postnatal maturation of the $[\text{Ca}^{2+}]_i$ response is linked to age-related differences in glomus cell depolarization in response to hypoxia. Glomus cells from newborns and mature rats have similar membrane potentials in normoxia, but cells from newborns depolarize significantly less in response to hypoxia. For a given level of hypoxic challenge, greater depolarization in mature glomus cells would result in greater Ca^{2+} influx through voltage-gated Ca^{2+} channels and could explain, in part, the previously reported increase in the $[\text{Ca}^{2+}]_i$ response to hypoxia during postnatal maturation (Sterni et al., 1995; Bamford et al., 1999; Wasicko et al., 1999). Although hypoxia-induced depolarization was $\sim 50\%$ less in cells from newborns compared to older rats, cells from both ages depolarize to the same degree in response to a non-hypoxic, non-specific depolarizing stimulus (high extracellular K^+ solution). Glomus cells from newborns and mature rats possess a background outward ionic current, largely carried by K^+ , which is inhibited by hypoxia less in glomus cells from newborns compared to mature rats. Thus, developmental changes in the hypoxia sensitivity of this background conductance could account, at least in part, for the age-related increase in glomus cell depolarization (and therefore $[\text{Ca}^{2+}]_i$ influx) in response to hypoxia. Taken together, these findings support the hypothesis that postnatal maturation of glomus cell O_2 sensitivity involves developmental regulation of the expression of and/or O_2 sensitivity of several ionic conductances.

4.1. Development of depolarization response to hypoxia

A developmental increase in the CB glomus cell $[\text{Ca}^{2+}]_i$ response to hypoxia could result from (a) an age-related increase in Ca^{2+} influx for a given degree of depolarization or (b) from an age-related increase

in the degree of depolarization for a given level of hypoxia. Because membrane depolarization and Ca^{2+} influx are critical steps in the O_2 chemosensory cascade, we hypothesized that postnatal maturation of the glomus cell $[\text{Ca}^{2+}]_i$ response to hypoxia might reflect age-related changes in hypoxia-induced depolarization.

Simultaneous recordings of $[\text{Ca}^{2+}]_i$ and E_m indicate that, compared to newborns, in glomus cells from older rats the magnitude of depolarization was ~ 2 times greater and the magnitude of the $[\text{Ca}^{2+}]_i$ increase was approximately 2–3 times greater in response to a standardized hypoxia stimulus. In both age groups, resting membrane potential during control (normoxia) conditions was in the range of -50 mV, consistent with previous reports (Hayashida and Hirakawa, 2002). Based on the current–voltage relation of neonatal rat glomus cell Ca^{2+} currents reported by Peers et al. (1996), a small developmental increase in the magnitude of hypoxia-induced depolarization could result in a large increase in the $[\text{Ca}^{2+}]_i$ response, due to the non-linear voltage dependence of Ca^{2+} influx.

The depolarization induced by 40 mM extracellular K^+ , which was of greater magnitude than the depolarization induced by hypoxia, was accompanied by a larger $[\text{Ca}^{2+}]_i$ response (Fig. 3), underscoring the close relationship between E_m depolarization and $[\text{Ca}^{2+}]_i$ in glomus cells. In addition, when glomus cells depolarize to approximately the same level (Fig. 3), the magnitude of the resulting increase in $[\text{Ca}^{2+}]_i$ is comparable in cells from newborn versus 11- to 14-day-old rats. These results suggest that the developmental increase in the glomus cell $[\text{Ca}^{2+}]_i$ response to hypoxia is due to developmental alterations in hypoxia-induced depolarization, rather than developmental regulation of $[\text{Ca}^{2+}]_i$ influx for a given degree of depolarization.

4.2. Development of background K^+ conductance response to hypoxia

Although the mechanism(s) of glomus cell depolarization by hypoxia are controversial, substantial evidence indicates that depolarization in response to hypoxia results from an inhibition of K^+ current (Lopez-Barneo et al., 1988; Delpiano and Hescheler, 1989; Peers, 1990; Ganfornina and Lopez-Barneo, 1991; Stea and Nurse, 1991; Buckler, 1997). Several O_2 sensitive K^+ currents have been characterized in

CB glomus cells, including a transient current sensitive to 4-AP (Lopez-Lopez et al., 1989), a voltage- and Ca^{2+} -activated, sustained current sensitive to charybdotoxin (Peers, 1990; Peers and O'Donnell, 1990), a voltage-activated, sustained current insensitive to charybdotoxin (Buckler, 1997), and a background (or 'leak') current insensitive to 4-AP, TEA and charybdotoxin (Buckler, 1997, 1999). The major candidates currently considered to be possible mediators of hypoxia-induced glomus cell depolarization in rats include large-conductance, Ca^{2+} -activated K^+ channels (K_{Ca}) (Peers, 1990) and non-voltage sensitive, background ("leak") K^+ channels (Buckler, 1997).

Although it is clear that glomus cells have background or "leak" K^+ conductance as originally described by Buckler (1997), the identity of the O_2 -sensitive channel(s) that initiate depolarization remains unknown. Available evidence suggests that glomus cells express several two-pore (2P) domain K^+ channels such as TASK-1, TASK-3 and TRAAK (Buckler, 1997; Buckler et al., 2000; Yamamoto et al., 2002; Williams and Buckler, 2004). The TASK-1 K^+ channel blocker methanandamide has been shown to reduce glomus cell resting conductance and inhibit an O_2 -sensitive background current (Wyatt and Buckler, 2003). Unfortunately, studies with this drug are problematic, as methanandamide is also a calcium channel antagonist (Wyatt and Buckler, 2003).

Barium inhibits background K^+ conductance by about 85% in glomus cells (Buckler, 1999). In this study, we compared the inhibition of background K^+ conductance by 5 mM Ba^{2+} to the inhibition caused by anoxia for both age groups. As shown in Fig. 4D, the O_2 -sensitive background current is similar in magnitude to the Ba^{2+} sensitive current, suggesting that most of the background current in the 11–14-day group is O_2 sensitive. In contrast, the O_2 -sensitive background current is only a small fraction of the Ba^{2+} sensitive current in glomus cells from 1- to 3-day-old rats (Fig. 4C). As shown in Table 1, the estimated O_2 -sensitive background conductance (control – anoxia) in glomus cells was 111 pS at 1–3 day versus 246 pS in the 11–14 day old group. With respect to CB development, the smaller magnitude of O_2 -sensitive background K^+ conductance in newborns may limit the ability of the glomus cell to initiate depolarization during hypoxia.

The magnitude of measured O_2 -sensitive background currents differed between the two protocols

employed. Using the ramp protocol (protocol 2) the O_2 -sensitive background current was estimated to be ~ 4 pA at -50 mV in 11–14-day-old rats (Fig. 4D). In contrast, as shown in Fig. 5, anoxia inhibited background current at -50 mV by ~ 12.5 pA in glomus cells of 11–14-day-old rats. The reason for this discrepancy is unknown. Data shown in Fig. 4 were measured using a ramp protocol in which V_m was increased steadily from -90 to -40 mV over 300 ms. In contrast the effect of graded hypoxia was measured, from a holding potential of -80 mV, using a pulse clamp protocol consisting of a series of 300 ms duration voltage steps in 10 mV increments between -60 and -20 mV. It is possible that differences in activation, inactivation or other aspects of channel kinetics differed between these two approaches. However, with respect to development, results from the two protocols yielded the same conclusion; the magnitude of the CB glomus cell O_2 -sensitive background conductance more than doubles between the newborn and ~ 2 weeks of age.

Using a pulse clamp protocol, we also examined the O_2 'sensitivity' of the background K^+ conductance by measuring the change in current in response to graded hypoxia. In cells from mature rats, as superfusate PO_2 was lowered, the background conductance was inhibited in a roughly hyperbolic manner (Fig. 5), as described by Buckler (1997), who studied 11–16-day-old rats. Developmental differences were observed at the more severe levels of superfusate PO_2 , i.e., the inhibition of the background K^+ current was $>50\%$ greater in the cells from mature rats compared to newborns. The effects of graded hypoxia on background K^+ currents shown in Fig. 5 are consistent with previously reported effects of graded hypoxia on the $[\text{Ca}^{2+}]_i$ response to hypoxia (Wasicko et al., 1999). In 11–21-day-old rats, the half-maximal $[\text{Ca}^{2+}]_i$ response to hypoxia was observed at a superfusate PO_2 of ~ 6 – 7 mmHg compared to cells from 1-day-old rats reaching half-maximal $[\text{Ca}^{2+}]_i$ response at ~ 2 mmHg PO_2 (see Fig. 5 in Wasicko et al., 1999). Fig. 5 of the present study indicates that background K^+ current was about half inhibited at ~ 7 – 8 mmHg in glomus cells from 11- to 14-day olds compared to newborn rats, which showed no inhibition until superfused with anoxic solution (Fig. 5). Therefore, the age-related PO_2 – E_m and PO_2 – $[\text{Ca}^{2+}]_i$ relationships for dissociated glomus cells in vitro are quite similar. Although these PO_2 values may seem low relative to arterial PO_2 , the

microvascular PO₂ in the cat carotid body has been reported to be considerably lower than arterial PO₂, with most neural activity occurring when tissue PO₂ falls below 15 mmHg (Rumsey et al., 1991).

4.3. Blockade of TEA/4-AP sensitive currents

The role of large-conductance calcium-activated K⁺ channels (K_{Ca}) in glomus cells is controversial. K_{Ca} channel antagonists have been shown cause catecholamine release in CB slices (Pardal et al., 2000; Pardal and Lopez-Barneo, 2002) and depolarization of dissociated glomus cells (Peers and Carpenter, 1998) while other studies have shown no effects of TEA, charybdotoxin or apamin on carotid body activity (Cheng and Donnelly, 1995). Hatton et al. have reported that K⁺ current density progressively increased with age in glomus cells isolated from 4, 10-day old and adult rats, primarily due to an age-related increase of O₂ sensitive, K_{Ca} channels (Hatton et al., 1997). Expression and activity of K_{Ca} channels, as well as K⁺ current sensitivity to hypoxia, increased between 4 and 10 days of age, with no further increase between 10 days and adults (Hatton et al., 1997), suggesting that K_{Ca} channels may contribute to postnatal maturation of O₂ sensing in the carotid body. The results shown in Fig. 6 indicate that 10 mM TEA and 5 mM 4-AP, which should block voltage-gated, calcium-activated (K_{Ca}), ATP-sensitive, KCNQ and possibly other K⁺ channels, did not initiate a [Ca²⁺]_i response but clearly increased the magnitude of the response to hypoxia at both ages. This suggests, at least for dissociated glomus cells, that TEA/4-AP-sensitive K⁺ channels normally serve to damp or limit the [Ca²⁺]_i response to hypoxia. TEA/4-AP increased the [Ca²⁺]_i response to hypoxia to the same extent in both age groups. In addition, the age-related increase in the [Ca²⁺]_i response to hypoxia persisted even when K_{Ca} and other TEA/4-AP-sensitive K⁺ channels were blocked.

4.4. Mechanisms of glomus cell hypoxia response maturation

The mechanisms by which hypoxia depolarizes glomus cells are complex, poorly understood, and controversial, and a full discussion is beyond the scope of the present study. Glomus cells express multiple 2P K⁺ channels and available evidence favors a TASK like

current as a major component of O₂-sensitive background K⁺ current in these cells (Wyatt and Buckler, 2003). The question of whether inhibition of a small-magnitude TASK-like O₂-sensitive background current is sufficient to initiate and drive glomus cell depolarization in response to hypoxia has been discussed elsewhere (Buckler, 1997, 1999). The main hypothesis of the present study was that postnatal maturation of the glomus cell [Ca²⁺]_i response to reduced PO₂ is due, at least in part, to development of mechanisms that initiate and determine the magnitude of hypoxia-induced depolarization. Our findings confirm that the magnitude of hypoxia-induced depolarization in enzymatically dissociated glomus cells more than doubles between birth and 14 days of age. In addition, the results confirm the presence of an O₂-sensitive background conductance in rat glomus cells, the magnitude of which approximately doubles between birth and ~2 weeks of age. Although this developmentally regulated background K⁺ current is remarkably similar to the TASK-like background conductance reported by Buckler (1997) with respect to magnitude and O₂ sensitivity, further study will be required to identify the channel(s) mediating this current. These studies are currently underway.

A postnatal increase in the O₂ sensitive K⁺ channel(s) that initiate and maintain depolarization is one mechanism for development of CB oxygen sensing. However, the glomus cell depolarization response to hypoxia could also increase with age if currents that limit or inhibit depolarization decrease with age. Another K⁺ conductance described in rat and rabbit glomus cells is a HERG-like K⁺ current (Overholt et al., 2000; Kim et al., 2005). HERG K⁺ channels (Human Ether-a-go-go Related Gene product) are potassium channels believed to play a major role in maintaining resting membrane potential and/or controlling repolarization in several excitable cell types, including neurosecretory cells (Bauer et al., 1998; Schafer et al., 1999; Pancrazio et al., 1999). HERG-like currents are present at immature stages of development and decline with age in other cell types (Arcangeli et al., 1997; Crociani et al., 2000). The carotid body is a neural crest-derived organ and HERG-like currents are expressed in tumors that develop in hypoxic microenvironments, particularly neural crest derived tumors (Arcangeli et al., 1997, 1999; D'Amico et al., 2003; Crociani et al., 2003). We recently reported that HERG-like K⁺ current density in glomus cells from 0- to 1-day-old

rats is two-fold greater than in cells from 11- to 16-day-old rats (Kim et al., 2005). These results suggest that membrane potential-stabilizing HERG-like K^+ currents are present in newborn CB glomus cells and decline with age, providing a potential mechanism for damping of excitability in newborn rat glomus cells that subsequently declines with age between birth and 14 days. Other K^+ channels that may serve a similar function are currently under investigation in our laboratory.

4.5. Intact carotid chemoreceptor maturation

This study focused on maturation of glomus cell depolarization and the $[Ca^{2+}]_i$ response to hypoxia. However, it is important to note that glomus cells exist in clusters, in contact with other glomus cells, type II cells and CSN nerve terminals. Glomus cells in situ are exposed to a complex microenvironment where a wide array of neurotransmitters, neuromodulators, changes in ion concentrations and pH may affect the resting state and/or the glomus cell response to hypoxia. Glomus cells express several subtypes of acetylcholine receptors, dopamine D2, 5-HT, P2Y, GABA_B, adenosine A2a and other receptors; CSN nerve terminals express additional receptors. Thus, apart from the maturation of glomus cell ionic current repertoire as described in the present study, differentially developing sensitivities of chemoreceptor cells to various neurotransmitters and neuromodulators also will contribute to shaping both the pre- and post-synaptic responses to hypoxia and any of these may also change during postnatal development.

Acknowledgements

This work was supported by National Institutes of Health Grant R01 HL54621 (JLC). Dr. Wasicko is currently with Goodell, DeVries, Leech and Dann, LLP, Baltimore, MD.

References

Arcangeli, A., Rosati, B., Cherubini, A., Crociani, O., Fontana, L., Ziller, C., Wanke, E., Olivotto, M., 1997. HERG- and IRK-like inward rectifier currents are sequentially expressed during neu-

ronal development of neural crest cells and their derivatives. *Eur. J. Neurosci.* 9, 2596–2604.

Arcangeli, A., Rosati, B., Crociani, O., Cherubini, A., Fontana, L., Passani, B., Wanke, E., Olivotto, M., 1999. Modulation of HERG current and herg gene expression during retinoic acid treatment of human neuroblastoma cells: potentiating effects of BDNF. *J. Neurobiol.* 40, 214–225.

Bamford, O.S., Sterni, L.M., Wasicko, M.J., Montrose, M.H., Carroll, J.L., 1999. Postnatal maturation of carotid body and type I cell chemoreception in the rat. *Am. J. Physiol.* 276, L875–L884.

Bauer, C.K., Engeland, B., Wulfsen, I., Ludwig, J., Pongs, O., Schwarz, J.R., 1998. RERG is a molecular correlate of the inward-rectifying K current in clonal rat pituitary cells. *Receptors Channels* 6, 19–29.

Biscoe, T.J., Purves, M.J., 1967. Carotid body chemoreceptor activity in the new-born lamb. *J. Physiol.* 190, 443–454.

Blanco, C.E., Dawes, G.S., Hanson, M.A., McCooke, H.B., 1984. The response to hypoxia of arterial chemoreceptors in fetal sheep and new-born lambs. *J. Physiol.* 351, 25–37.

Buckler, K.J., 1999. Background leak K^+ -currents and oxygen sensing in carotid body type I cells. *Respir. Physiol.* 115, 179–187.

Buckler, K.J., 1997. A novel oxygen-sensitive potassium current in rat carotid body type I cells. *J. Physiol.* 498 (Pt 3), 649–662.

Buckler, K.J., Vaughan-Jones, R.D., 1994. Effects of hypoxia on membrane potential and intracellular calcium in rat neonatal carotid body type I cells. *J. Physiol.* 476, 423–428.

Buckler, K.J., Williams, B.A., Honore, E., 2000. An oxygen-, acid- and anaesthetic-sensitive TASK-like background potassium channel in rat arterial chemoreceptor cells. *J. Physiol.* 525 (Pt 1), 135–142.

Bureau, M.A., Lamarche, J., Foulon, P., Dalle, D., 1985b. The ventilatory response to hypoxia in the newborn lamb after carotid body denervation. *Respir. Physiol.* 60, 109–119.

Bureau, M.A., Lamarche, J., Foulon, P., Dalle, D., 1985a. Postnatal maturation of respiration in intact and carotid body-chemodenedervated lambs. *J. Appl. Physiol.* 59, 869–874.

Carroll, J.L., Bamford, O.S., Fitzgerald, R.S., 1993. Postnatal maturation of carotid chemoreceptor responses to O_2 and CO_2 in the cat. *J. Appl. Physiol.* 75, 2383–2391.

Cheng, P.M., Donnelly, D.F., 1995. Relationship between changes of glomus cell current and neural response of rat carotid body. *J. Neurophysiol.* 74, 2077–2086.

Cote, A., Porras, H., Meehan, B., 1996. Age-dependent vulnerability to carotid chemodervation in piglets. *J. Appl. Physiol.* 80, 323–331.

Crociani, O., Cherubini, A., Piccini, E., Polvani, S., Costa, L., Fontana, L., Hofmann, G., Rosati, B., Wanke, E., Olivotto, M., Arcangeli, A., 2000. Erg gene(s) expression during development of the nervous and muscular system of quail embryos. *Mech. Dev.* 95, 239–243.

Crociani, O., Guasti, L., Balzi, M., Becchetti, A., Wanke, E., Olivotto, M., Wymore, R.S., Arcangeli, A., 2003. Cell cycle-dependent expression of HERG1 and HERG1B isoforms in tumor cells. *J. Biol. Chem.* 278, 2947–2955.

D'Amico, M., Biagiotti, T., Fontana, L., Restano-Cassulini, R., Lasagna, N., Arcangeli, A., Wanke, E., Olivotto, M., 2003. A HERG current sustains a cardiac-type action potential in neu-

- roblastoma S cells. *Biochem. Biophys. Res. Commun.* 302, 101–108.
- Delpiano, M.A., Hescheler, J., 1989. Evidence for a PO₂-sensitive K⁺ channel in the type-I cell of the rabbit carotid body. *FEBS Lett.* 249, 195–198.
- Donnelly, D.F., Haddad, G.G., 1990. Prolonged apnea and impaired survival in piglets after sinus and aortic nerve section. *J. Appl. Physiol.* 68, 1048–1052.
- Fewell, J.E., Kondo, C.S., Dascalu, V., Filyk, S.C., 1989a. Influence of carotid denervation on the arousal and cardiopulmonary response to rapidly developing hypoxemia in lambs. *Pediatr. Res.* 25, 473–477.
- Fewell, J.E., Kondo, C.S., Dascalu, V., Filyk, S.C., 1989b. Influence of carotid-denervation on the arousal and cardiopulmonary responses to alveolar hypercapnia in lambs. *J. Dev. Physiol.* 12, 193–199.
- Fewell, J.E., Taylor, B.J., Kondo, C.S., Dascalu, V., Filyk, S.C., 1990. Influence of carotid denervation on the arousal and cardiopulmonary responses to upper airway obstruction in lambs. *Pediatr. Res.* 28, 374–378.
- Ganformina, M.D., Lopez-Barneo, J., 1991. Single K⁺ channels in membrane patches of arterial chemoreceptor cells are modulated by O₂ tension. *Proc. Natl. Acad. Sci. U.S.A.* 88, 2927–2930.
- Gonzalez, C., Almaraz, L., Obeso, A., Rigual, R., 1994. Carotid body chemoreceptors: from natural stimuli to sensory discharges. *Physiol. Rev.* 74, 829–898.
- Grynkiewicz, G., Poenie, M., Tsien, R.Y., 1985. A new generation of Ca²⁺ indicators with greatly improved fluorescence properties. *J. Biol. Chem.* 260, 3440–3450.
- Hatton, C.J., Carpenter, E., Pepper, D.R., Kumar, P., Peers, C., 1997. Developmental changes in isolated rat type I carotid body cell K⁺ currents and their modulation by hypoxia. *J. Physiol.* 501 (Pt 1), 49–58.
- Hayashida, Y., Hirakawa, H., 2002. Electrical properties of chemoreceptor elements in the carotid body. *Microsc. Res. Tech.* 59, 243–248.
- Hofer, M.A., 1984. Lethal respiratory disturbance in neonatal rats after arterial chemoreceptor denervation. *Life Sci.* 34, 489–496.
- Hofer, M.A., 1986. Role of carotid sinus and aortic nerves in respiratory control of infant rats. *Am. J. Physiol.* 251, R811–R817.
- Kholwadwala, D., Donnelly, D.F., 1992. Maturation of carotid chemoreceptor sensitivity to hypoxia: in vitro studies in the newborn rat. *J. Physiol.* 453, 461–473.
- Kim, I., Boyle, K.M., Carroll, J.L., 2005. Postnatal development of E-4031-sensitive potassium current in rat carotid chemoreceptor cells. *J. Appl. Physiol.* 98, 1469–1477.
- Lopez-Barneo, J., Lopez-Lopez, J.R., Urena, J., Gonzalez, C., 1988. Chemotransduction in the carotid body: K⁺ current modulated by PO₂ in type I chemoreceptor cells. *Science* 241, 580–582.
- Lopez-Lopez, J., Gonzalez, C., Urena, J., Lopez-Barneo, J., 1989. Low pO₂ selectively inhibits K⁺ channel activity in chemoreceptor cells of the mammalian carotid body. *J. Gen. Physiol.* 93, 1001–1015.
- Marchal, F., Bairam, A., Haouzi, P., Crance, J.P., Di Giulio, C., Vert, P., Lahiri, S., 1992. Carotid chemoreceptor response to natural stimuli in the newborn kitten. *Respir. Physiol.* 87, 183–193.
- Mulligan, E., Lahiri, S., 1987. Cat carotid body chemoreceptor responses before and after nicotine receptor blockade with alpha-bungarotoxin. *J. Auton. Nerv. Syst.* 18, 25–31.
- Nurse, C.A., 1990. Carbonic anhydrase and neuronal enzymes in cultured glomus cells of the carotid body of the rat. *Cell Tissue Res.* 261, 65–71.
- Overholt, J.L., Ficker, E., Yang, T., Shams, H., Bright, G.R., Prabhakar, N.R., 2000. HERG-like potassium current regulates the resting membrane potential in glomus cells of the rabbit carotid body. *J. Neurophysiol.* 83, 1150–1157.
- Pancrazio, J.J., Ma, W., Grant, G.M., Shaffer, K.M., Kao, W.Y., Liu, Q.Y., Manos, P., Barker, J.L., Stenger, D.A., 1999. A role for inwardly rectifying K⁺ channels in differentiation of NG108-15 neuroblastoma × glioma cells. *J. Neurobiol.* 38, 466–474.
- Pang, L., Eyzaguirre, C., 1992. Different effects of hypoxia on the membrane potential and input resistance of isolated and clustered carotid body glomus cells. *Brain Res.* 575, 167–173.
- Pardal, R., Lopez-Barneo, J., 2002. Carotid body thin slices: responses of glomus cells to hypoxia and K⁺-channel blockers. *Respir. Physiol. Neurobiol.* 132, 69–79.
- Pardal, R., Ludewig, U., Garcia-Hirschfeld, J., Lopez-Barneo, J., 2000. Secretory responses of intact glomus cells in thin slices of rat carotid body to hypoxia and tetraethylammonium. *Proc. Natl. Acad. Sci. U.S.A.* 97, 2361–2366.
- Peers, C., 1990. Hypoxic suppression of K⁺ currents in type I carotid body cells: selective effect on the Ca²⁺-activated K⁺ current. *Neurosci. Lett.* 119, 253–256.
- Peers, C., Carpenter, E., 1998. Inhibition of Ca²⁺-dependent K⁺ channels in rat carotid body type I cells by protein kinase C. *J. Physiol.* 512 (Pt 3), 743–750.
- Peers, C., Carpenter, E., Hatton, C.J., Wyatt, C.N., Bee, D., 1996. Ca²⁺ channel currents in type I carotid body cells of normoxic and chronically hypoxic neonatal rats. *Brain Res.* 739, 251–257.
- Peers, C., O'Donnell, J., 1990. Potassium currents recorded in type I carotid body cells from the neonatal rat and their modulation by chemoexcitatory agents. *Brain Res.* 522, 259–266.
- Rumsey, W.L., Iturriaga, R., Spergel, D., Lahiri, S., Wilson, D.F., 1991. Optical measurements of the dependence of chemoreception on oxygen pressure in the cat carotid body. *Am. J. Physiol.* 261, C614–C622.
- Schafer, R., Wulfsen, I., Behrens, S., Weinsberg, F., Bauer, C.K., Schwarz, J.R., 1999. The erg-like potassium current in rat lactotrophs. *J. Physiol.* 518 (Pt 2), 401–416.
- Stea, A., Nurse, C.A., 1991. Whole-cell and perforated-patch recordings from O₂-sensitive rat carotid body cells grown in short- and long-term culture. *Pflugers Arch.* 418, 93–101.
- Sterni, L.M., Bamford, O.S., Tomares, S.M., Montrose, M.H., Carroll, J.L., 1995. Developmental changes in intracellular Ca²⁺ response of carotid chemoreceptor cells to hypoxia. *Am. J. Physiol.* 268, L801–L808.
- Wasicko, M.J., Sterni, L.M., Bamford, O.S., Montrose, M.H., Carroll, J.L., 1999. Resetting and postnatal maturation of oxygen chemosensitivity in rat carotid chemoreceptor cells. *J. Physiol.* 514 (Pt 2), 493–503.

- Williams, B.A., Buckler, K.J., 2000. Identification of an oxygen-sensitive potassium channel in neonatal rat carotid body type I cells. *Adv. Exp. Med. Biol.* 475, 419–424.
- Williams, B.A., Buckler, K.J., 2004. Biophysical properties and metabolic regulation of a TASK-like potassium channel in rat carotid body type I cells. *Am. J. Physiol. Lung Cell Mol. Physiol.* 286, L221–L230.
- Wyatt, C.N., Buckler, K.J., 2003. The effect of methanandamide on isolated type I cells. *Adv. Exp. Med. Biol.* 536, 123–127.
- Yamamoto, Y., Kummer, W., Atoji, Y., Suzuki, Y., 2002. TASK-1, TASK-2, TASK-3 and TRAAK immunoreactivities in the rat carotid body. *Brain Res.* 950, 304.