Long-Term Modulation of Inward Currents in O₂ Chemoreceptors by Chronic Hypoxia and Cyclic AMP *in vitro*

A. Stea,^a A. Jackson, L. Macintyre, and C. A. Nurse

Department of Biology, McMaster University, Hamilton, Ontario, Canada, L8S 4K1

In mammals, ventilatory acclimatization to hypoxia is associated with an enhanced chemosensitivity of the O₂-sensing carotid body, resulting in an increased respiratory drive. To test whether this sensitization involves long-term modulation of ion channel function in endogenous O₂ chemoreceptors, i.e., type 1 cells, we exposed cultures of dissociated rat carotid body to chronic hypoxia (6% O₂) for 1–2 weeks, before monitoring the electrophysiological properties of type 1 cells using whole-cell, perforated patch recording. Chronic hypoxia augmented voltage-dependent inward Na⁺ and Ca²⁺ currents in type 1 cells, without significant changes in voltage dependence of activation or steady-state inactivation. However, after normalizing for the concomitant increase in cell size, indicated by the whole-cell capacitance, only the Na⁺ current density was significantly enhanced. The Na⁺ current was sensitive to tetrodotoxin (TTX; 0.5–1 μM) or choline substitution, whereas most of the Ca²⁺ current was sensitive to the L-type calcium channel blocker, nifedipine (10 μM). Several of these effects of hypoxia were mimicked qualitatively by growing normoxic cultures in the presence of agents that elevate intracellular cyclic AMP, including dibutyryl cAMP (db-cAMP; 200 μM–1 mM) and forskolin (10 μM); treatment with similar concentrations of dibutyryl cyclic GMP was ineffective. Na⁺ channel induction by db-cAMP was abolished by the protein synthesis inhibitor, cycloheximide (90–180 μM). In current-clamp mode, these altered chemoreceptors had typical resting potentials of ~ -55 mV, and following depolarization often fired multiple spikes that appeared to consist of both short-duration Na⁺ and long-duration Ca²⁺ components. We propose that chronic hypoxia, acting in part through cAMP-dependent pathways, increases electrical excitability and calcium mobilization in type 1 cells, and these adaptations may help enhance chemosensitivity during hypoxic acclimatization.

[Key words: glomus cell, chronic hypoxia in vitro, chemoreceptor, adaptation, cAMP analogs, Na⁺ channel induction, Ca²⁺ channel modulation]

In mammals, the carotid body is a major oxygen-sensing organ that regulates breathing and is primarily responsible for the hyperventilation during acute hypoxia (Eyzaguirre and Zapata, 1984; Biscoe and Duchon, 1990; Gonzalez et al., 1992). It is now generally accepted that the endogenous glomus or type 1 cells are the transducers of hypoxic stimuli, and relay chemosensory information to the brainstem via neurotransmitter release at synaptic contacts with afferent terminals of the carotid sinus nerve. Though the mechanisms of O₂ chemotransduction remain controversial, the popular view is that closure of oxygen-sensitive K⁺ channels in the plasma membrane of type 1 cells is an important event in hypoxic chemotransduction, facilitating cell depolarization, calcium entry, and neurotransmitter release (Gonzalez et al., 1992; Lopez-Barneo et al., 1993; see, however, Biscoe and Duchon, 1990). Though regulation of these K⁺ channels by acute hypoxia can occur in membrane patches, apparently without second-messenger involvement (Ganforina and Lopez-Barneo, 1991, 1992; see, however, Peers, 1990), the possibility that cyclic nucleotides may yet play a physiological role in O₂ sensing has not been excluded (Gonzalez et al., 1992; Lopez-Lopez et al., 1993). Significantly, acute hypoxia increases cAMP levels in the carotid body in a dose-dependent manner (Perez-Garcia et al., 1990; Delpiano and Acker, 1991), and this increase appears to be specific to type 1 cells (Wang et al., 1991).

We are interested in the adaptive capabilities of type 1 cells following chronic exposure to low oxygen, and particularly the role of cyclic nucleotides as mediators in the long-term regulation of ion channel function during hypoxia. Conceivably, such regulation of ion channels could be physiologically important in the sensitization of the carotid body response, which is known to occur during the period of ventilatory acclimatization of animals and humans to chronic low pO₂, for example, high altitude (Barnard et al., 1987; Nielsen et al., 1988). Using dissociated cell cultures of the rat carotid body, we recently showed that chronic hypoxia increased Na⁺ (but not K⁺) current density in type 1 cells, and that the effect was mimicked by chronic treatment with dibutyryl cAMP (Stea et al., 1992). However, in the latter study, no consideration was given to the possible involvement of calcium channel currents, which could also participate in the increased chemoreceptor sensitivity during hypoxic acclimatization, via modulation of both electrical excitability and neurotransmitter release from type 1 cells. Indeed, L-type Ca²⁺ channels, which are the predominant calcium channel type in these cells (Urena et al., 1989; Peers, 1990; Fieber and Mc-

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Correspondence should be addressed to Dr. C. A. Nurse, Department of Biology, McMaster University, 1280 Main St. West, Hamilton, Ontario, Canada L8S 4K1.

^aPresent address: Biotechnology Laboratory, Room 237-Wesbrook Building, 6174 University Boulevard, University of British Columbia, Vancouver, B.C., Canada V6T 1Z3.

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Cleskey, 1993; Buckler and Vaughan-Jones, 1994), are known to be induced by cAMP in other cell types, for example, astrocytes (Barres et al., 1989), and modulated by cAMP-dependent protein kinase and other signal transduction pathways (for references, see Hess, 1990; Yoshida et al., 1992).

The aims of the present study were to obtain a more complete understanding of the physiological mechanisms underlying chemoreceptor plasticity during chronic stimulation by low pO_2 . Particular points of interest relevant to type 1 cells were as follows. (1) Does chronic hypoxia or cAMP elevation cause significant changes in activation or inactivation properties of the augmented Na^+ current? (2) Is there a hypoxia-dependent modulation of calcium currents, that could also be mediated via elevation of intracellular cAMP? (3) Can the induction of Na^+ channels be mimicked by pharmacological activation of an endogenous adenylate cyclase? (4) Is there specificity among the cyclic nucleotides (cAMP vs cGMP) in the induction of Na^+ (and possibly Ca^{2+}) channels? (5) Is protein synthesis required for the induction of Na^+ (and possibly Ca^{2+}) channels, as occurs in other systems where cAMP promotes long-term upregulation of ion channels (Offord and Catterall, 1989)? (6) How is electrical excitability altered after chronic hypoxia or cAMP elevation? We address these questions in dispersed cell cultures of the rat carotid body, where type 1 cells can survive for several weeks in a controlled low O_2 atmosphere, free from secondary *in vivo* circulatory influences.

Materials and Methods

Culture conditions. Primary cultures of dissociated rat carotid bodies were prepared as described in detail elsewhere (Nurse, 1987; Stea and Nurse, 1991). Briefly, carotid bodies were dissected from neonatal rat pups, 5–12 d old (Wistar, Charles River, Quebec), followed by incubation for 1 hr in an enzymatic solution containing 0.1% trypsin, 0.1% collagenase (GIBCO, Grand Island, NY), and 0.01% deoxyribonuclease (Millipore Corp., Freehold, NJ). The tissues were then mechanically dissociated and the resulting cell suspension plated into collagen-coated, central wells of modified 35 mm petri dishes. The growth medium consisted of F-12 nutrient medium (GIBCO) supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 0.6% glucose, 1% penicillin-streptomycin (GIBCO), and 80 U/liter insulin (Sigma Chemical Co.; St. Louis, MO). The cultures were incubated at 37°C in a humidified atmosphere of 95% air:5% CO_2 for 2 d before exposing them to one of the following treatments for an additional 3–15 d: (1) a control normoxic environment (20% O_2 /75% N_2 /5% CO_2); (2) a hypoxic environment (6% O_2 /89% N_2 /5% CO_2); (3) a normoxic environment with either dibutyryl cyclic AMP (db-cAMP; 200–1000 μM), or dibutyryl cyclic GMP (db-cGMP; 200 μM), or forskolin (10 μM) present in the growth medium.

In a set of experiments designed to test whether protein synthesis was required for the db-cAMP-mediated increase in Na^+ channel density in glomus cells, the protein synthesis inhibitor cycloheximide was used. In these studies, 2-d-old carotid body cultures were divided into three groups and incubated under normoxic conditions for a further 3–4 d in one of the following media: (1) control growth medium; (2) control medium plus 1 mM db-cAMP; or (3) control medium plus 1 mM db-cAMP, but with 90–180 μM cycloheximide present for the first 36–48 hr. Longer treatment with cycloheximide resulted in significant toxicity to the cultures.

Whole-cell recording and data analysis. In most experiments, the perforated-patch technique was used for whole-cell recording from glomus cells as described previously (Stea and Nurse, 1991; Stea et al., 1992). Briefly, the antibiotic nystatin (Sigma) was dissolved in dimethyl sulfoxide (DMSO) and added to the pipette solution at a final concentration of 500 $\mu g/ml$. Within 5–10 min the series resistance decreased typically to 10–15 M Ω and was compensated in most experiments before recording of whole-cell currents. Voltage- or current-clamp records were obtained with a Dagan 3900 or Axopatch 1D patch clamp amplifier equipped with a 1 G Ω headstage feedback resistor, digitized with the aid of a computer interface (Axolab 1100 or Labmaster TL-1; Axon

Instruments, Foster City, CA) and stored on disk in an IBM-compatible 386 computer using pCLAMP version 5.5 software (Axon Instruments). Input capacitance was determined by integration of the capacity current transient elicited during a voltage step from -60 to -120 mV and dividing the resulting charge by the voltage step. To calculate the Na^+ or Ca^{2+} current density, peak currents elicited by voltage steps from -60 or -100 mV to 0 mV were divided by the input capacitance. In a few experiments the effect of cycloheximide on K^+ current density was tested; here, steady-state K^+ currents elicited during a step from -60 to $+50$ mV (after ~ 30 msec) were divided by the input capacitance.

Voltage dependence of channel activation was determined from current-voltage relations, generated during voltage steps from a holding potential of -80 mV to various test potentials. Peak currents were measured for each test potential after 3–5 msec for Na^+ currents and after 20–30 msec for Ca^{2+} currents. These data points were fitted with a smooth curve according to the equation

$$I = G \times (V - E) / [1 + \exp(V - V_{1/2})/k],$$

where I is the peak current at a given test potential V , G is the conductance, E is the reversal potential, $V_{1/2}$ is the voltage for half-activation, and k is the slope factor. Values of $V_{1/2}$ are given in Table 1.

Steady-state inactivation curves were generated from the peak inward currents elicited by steps to 0 mV after holding the cell at various potentials for >10 sec. These data were fitted with smooth curves according to a modified version of the Boltzmann equation:

$$h = \frac{I_{Na}(V_m)}{I_{Na,max}} = \frac{1}{\exp\left(\frac{V_m - V_{1/2}}{S_{1/2}}\right) + 1},$$

where h is the steady-state inactivation determined by dividing the peak I_{Na} at each holding potential (V_m) by the maximum I_{Na} recorded. The variables $V_{1/2}$ (holding potential where I_{Na} was half maximal) and $S_{1/2}$ (slope factor at $V_{1/2}$) were adjusted for best fit of the curve to the observed data. Values of $V_{1/2}$ were calculated from steady-state inactivation curves for the fast transient Na^+ currents and the slower, prolonged inward Ca^{2+} currents in glomus cells under various treatments.

Significant differences between various parameters versus control were determined with a Student's t test with the significance level set at $p < 0.01$. Records shown in the figures were plotted with a Hewlett-Packard Laserjet III printer after subtraction of capacitive and leakage currents (except where noted) and filtering at 1000–2000 Hz.

Solutions. The majority of voltage-clamp recordings described below was obtained with extracellular fluid of the following composition (mM): NaCl, 135; KCl, 5; $CaCl_2$, 2; $MgCl_2$, 2; glucose, 10; N -2-hydroxyethylpiperazine- N' -2-ethane sulfonic acid (HEPES), 10 at pH 7.4. Pipette solutions used to record Ca^{2+} currents (with K^+ currents blocked) typically contained (mM) CsCl, 105; tetraethyl ammonium chloride (TEA), 25; NaCl, 10; ethylene glycol-bis (β -aminoethyl ether)- N,N,N',N' -tetraacetic acid (EGTA), 11; $CaCl_2$, 1; HEPES, 10 at pH 7.2. When K^+ currents were monitored, the pipette contained (mM) KCl, 135; NaCl, 5; $CaCl_2$, 0.1; HEPES, 10 at pH 7.2. In several experiments, to reduce osmotic disturbances during perforated-patch recording, a low Cl^- pipette solution with the following composition (mM) was used: CsCl, 30; TEA acetate, 20; Cs_2SO_4 , 45; Na gluconate, 5; sucrose, 55; $CaCl_2$, 0.1; HEPES, 10 at pH 7.2. Since no differences were obvious between recordings in high and low Cl^- , data were pooled and described below.

Drugs were added to the extracellular solution to block selective ionic currents. In a few experiments, tetrodotoxin (TTX; Sigma) was added at a final concentration of 0.2 to 1 μM to block voltage-activated Na^+ currents and tetraethylammonium (TEA) chloride at 5 mM to block K^+ currents. In some cases, whole-cell Na^+ currents were eliminated by equimolar substitution of extracellular Na^+ with (impermeant) choline. Calcium currents were blocked by equimolar substitution of cobalt for extracellular calcium in some experiments; in others, selective blockers for L- and N-type Ca^{2+} channels, i.e., nifedipine and ω -conotoxin GVIA (Research Biochemicals Inc., Natick, MA), respectively, were used. Nifedipine was prepared as a 10 mM stock solution in DMSO and used at a final concentration of 10 μM ; ω -conotoxin GVIA was prepared as a 100 μM stock solution in distilled water and used at a final concentration of 1 μM . All recordings were done at room temperature. Junction potentials, typically in the range 4–10 mV, were canceled at the beginning of the experiment.

Table 1. Effects of chronic treatments on electrophysiological characteristics of glomus cells

Treatment ^a	$I_{Na,max}^b$ (pA)	$I_{Ca,max}^b$ (pA)	Act. $V_{1/2,Na}^c$ (mV)	Act. $V_{1/2,Ca}^c$ (mV)	Inact. $V_{1/2,Na}^d$ (mV)	Inact. $V_{1/2,Ca}^d$ (mV)	C_{in}^e (pF)
Control	82.8 ± 22.7 (<i>n</i> = 8)	31.3 ± 3.9 (<i>n</i> = 8)	−19.3 ± 2.8 (<i>n</i> = 4)	−7.6 ± 4.8 (<i>n</i> = 6)	−53.5 ± 1.4 (<i>n</i> = 3)	−38.7 ± 2.7 (<i>n</i> = 5)	8.9 ± 0.6 (<i>n</i> = 22)
Hypoxia (6% O ₂)	444.6* ± 64.2 (<i>n</i> = 10)	101.0* ± 15.4 (<i>n</i> = 9)	−24.9 ± 1.9 (<i>n</i> = 9)	−5.6 ± 4.1 (<i>n</i> = 10)	−48.0 ± 3.0 (<i>n</i> = 6)	−32.6 ± 1.3 (<i>n</i> = 6)	20.4* ± 2.2 (<i>n</i> = 15)
db-cAMP (1 mM)	458.3* ± 89.4 (<i>n</i> = 14)	37.1 ± 5.0 (<i>n</i> = 14)	−24.0 ± 2.2 (<i>n</i> = 6)	−11.2 ± 3.1 (<i>n</i> = 6)	−59.7 ± 2.3 (<i>n</i> = 6)	−33.2 ± 1.9 (<i>n</i> = 5)	11.7* ± 0.9 (<i>n</i> = 22)
Forskolin (10 μM)	618.5* ± 255.0 (<i>n</i> = 8)	70.5* ± 9.4 (<i>n</i> = 8)	−17.6 ± 1.8 (<i>n</i> = 9)	−7.6 ± 2.9 (<i>n</i> = 9)	−50.4 ± 3.2 (<i>n</i> = 4)	−39.8 ± 1.9 (<i>n</i> = 2)	15.6* ± 1.5 (<i>n</i> = 9)

^a Treatment period lasted 9–14 d.^b Mean values ± SEM for voltage steps from −100 to 0 mV measured after 2–5 msec for Na⁺ currents and 20–30 msec for Ca²⁺ currents.^c Mean values ± SEM determined from equation used to fit *I/V* curves as in Figure 2C (see Materials and Methods).^d Mean values ± SEM determined from steady-state inactivation curves as in Figure 2D (see Materials and Methods).^e Mean values ± SEM determined by integration of the capacity current transient divided by the voltage step.* Significant from controls *p* < 0.01.

Results

Modulation of inward currents in glomus cells by chronic hypoxia

We previously showed that treatment of dissociated rat carotid body cultures with chronic hypoxia (6% O₂) caused a significant increase in peak inward Na⁺ current in glomus cells (Stea et al., 1992). After normalizing for the concomitant increase in cell size, estimated from the whole-cell capacitance, the net effect was a selective increase in Na⁺ current density; the K⁺ current density was unchanged or tended to decrease at longer exposure times. In the present study, the K⁺ currents were blocked in many experiments by substitution with Cs⁺ in the pipette (see Materials and Methods), and this allowed a direct comparison of the long-term effects of hypoxia on the macroscopic inward Na⁺ and Ca²⁺ currents. Since perforated-patch recording was used, thereby restricting intracellular dialysis (Horn and Marty, 1988), calcium currents were relatively stable and intracellular Ca²⁺ concentration was determined mainly by the cell's intrinsic buffering. As shown in Figure 1 (upper panel), the two components of inward current, elicited by a voltage step from −60 mV to 0 mV, were both enlarged in glomus cells exposed to ~2 weeks of chronic hypoxia compared to control (normoxia). The fast, rapidly inactivating component is primarily a Na⁺ current, since it was eliminated or substantially reduced following substitution of external Na⁺ with the impermeant choline, without effect on the slower component of inward current (*n* = 8; Fig. 2A). In addition, this fast component was almost entirely abolished by 0.5–1 μM tetrodotoxin, a selective blocker of voltage-gated Na⁺ channels (*n* = 3; not shown), as we previously reported (Stea et al., 1992). The other, sustained or slowly inactivating component of inward current (Fig. 1) is carried by Ca²⁺ ions since it was almost entirely eliminated by substituting 2 mM cobalt for extracellular Ca²⁺, with little effect on the fast component (*n* = 11; Fig. 2A).

The long-term effects of hypoxia on peak components of inward current were substantial. Over a 2-week exposure period, the peak transient Na⁺ current increased ~ eightfold, from 43.4 ± 5.8 pA (mean ± SE, *n* = 22; normoxia) to 345.3 ± 50.3

pA (*n* = 17; hypoxia), for a voltage step from −60 mV (i.e., near the estimated resting potential; see later) to 0 mV. The corresponding increase for the prolonged, slowly inactivating Ca²⁺ current was ~ threefold, from 26.1 ± 3.5 pA (*n* = 22; normoxia) to 71.9 ± 8.6 pA (*n* = 15). Steps from a more hyperpolarized holding potential (−100 mV), where inactivation was removed, revealed maximal inward current as indicated in Table 1. However, as discussed later, only the increase in Na⁺ current appeared to involve a specific inductive effect; the increase in Ca²⁺ current was apparently due to the concomitant increase in cell size, indicated by an increase in input capacitance *C_{in}* (see Table 1). Moreover, with respect to these inward currents, chronic hypoxia did not cause any major shift in activation threshold or voltage dependence of channel activation and inactivation. In hypoxia-treated cells, the activation threshold for the inward currents occurred around −40 mV (Fig. 2C), similar to that for control cells (not shown; see Urena et al., 1989; Stea and Nurse, 1991; Stea et al., 1992). In addition, a comparison of *I-V* curves elicited from a holding potential of −80 mV for chronically hypoxic and control normoxic cells showed no significant differences in the voltage for half-activation (*V*_{1/2}) for either the Na⁺ or Ca²⁺ current (see Table 1 and Materials and Methods). Typical examples of the *I-V* relation for the inward Na⁺ and Ca²⁺ currents in glomus cells treated for 2 weeks in 6% O₂ are shown in Figure 2C. Analysis of the steady-state inactivation curves (see Materials and Methods) indicated that for hypoxia-treated cells the holding potential or prepulse voltage resulting in half-maximal peak current (*V*_{1/2}) was not significantly different from controls for Ca²⁺ currents, and though the mean value of *V*_{1/2} for Na⁺ currents appeared to shift to a more positive potential, it was not significant (Table 1). Further, this apparent shift was insufficient to explain the hypoxia-induced increase in peak inward Na⁺ current (Fig. 1, upper right), since when maximal Na⁺ currents were evoked during steps to 0 mV from large negative prepulse potentials (−100 mV), there was still a more than fivefold increase in peak Na⁺ current in hypoxia-treated cells (Table 1). The simplest explanation is that the

Figure 1. Effect of chronic treatments on whole-cell inward currents of carotid body type 1 (glomus) cells. Cultured glomus cells exposed to hypoxia (6% O₂), 1 mM db-cAMP, or 10 μ M forskolin for 8–15 d showed larger inward currents than control normoxic cells. Whole-cell currents recorded with a K⁺-free pipette solution (see Materials and Methods) consist of a large, rapidly inactivating Na⁺ component and a smaller, slowly inactivating Ca²⁺ component. Voltage step, -60 to 0 mV.

increase in Na⁺ current following chronic hypoxia is due to an increased expression of functional Na⁺ channels (see later).

Effect of chronic elevation of cyclic nucleotides

Exposure of the carotid body to hypoxia is known to regulate differentially the levels of intracellular second messengers, cAMP and cGMP, in glomus cells (Wang et al., 1991). Since these cyclic nucleotides promote short- and long-term modulation of ion channel function in a variety of cell types (Barres et al., 1989; Offord and Catterall, 1989; Kalman et al., 1990; Pollock et al., 1990; Dolphin, 1991), it was of interest to investigate whether they could mimic the above effects of chronic hypoxia on whole-cell currents.

Cyclic AMP elevation. As shown in Figure 1 (lower panel), chronic elevation of intracellular cAMP following treatment with either dibutyl cAMP (db-cAMP; 500–1000 μ M) or an activator of adenylate cyclase (forskolin; 10 μ M) for 8–15 d, mimicked the effects of hypoxia in augmenting both the fast transient Na⁺, and prolonged Ca²⁺ inward currents. Regardless of the treatment, the transient component was abolished by substituting choline for Na⁺ in the bath ($n = 7$, db-cAMP treatment; $n = 4$, forskolin treatment), or by the addition of 0.5–1 μ M TTX ($n = 4$, db-cAMP; $n = 2$, forskolin). Similarly, in all cases tested the prolonged Ca²⁺ component was blocked by substituting Ca²⁺ with 2 mM cobalt ($n = 9$, db-cAMP; $n = 6$, forskolin). Figure 2B is an example illustrating selective blockade of each component of inward current in a glomus cell treated for 13 d with 10 μ M forskolin.

As was the case for chronic hypoxia, the effects of chronic cAMP elevation on inward currents appeared to be due to an

increase in number of functional channels rather than to major shifts in activation properties or steady-state inactivation. Compared to controls (see above), the maximum Na⁺ current evoked during steps to 0 mV from hyperpolarized holding potentials (-100 mV) was more than fivefold higher after chronic treatment with 1 mM db-cAMP, and more than sevenfold higher with 10 μ M forskolin (Table 1). For Ca²⁺ currents the maximum levels were 1.2- to 2.3-fold higher than controls for db-cAMP and forskolin treatments (Table 1). The activation thresholds and $V_{1/2}$ values for Na⁺ and Ca²⁺ current, as well as the $V_{1/2}$ for steady-state inactivation, were unaffected by either treatment (Table 1). Comparison of steady-state inactivation curves for the Na⁺ and Ca²⁺ currents in a glomus cell treated for 8 d with 1 mM db-cAMP is shown in Figure 2D, where the $V_{1/2}$ for the Ca²⁺ current was shifted by ~ 25 mV to the right relative to that for the Na⁺ current.

Cyclic GMP elevation. Exposure of glomus cells to 200–1000 μ M db-cGMP, the membrane permeant analog of cGMP, for 8–15 d did not significantly affect either component of inward current nor cell size, as indicated by the whole-cell capacitance. The latter values were 10.4 ± 1.0 pF ($n = 13$) in 200 μ M db-cGMP and 7.7 ± 1.6 pF ($n = 4$) in 1 mM db-cGMP, which are similar to controls (Table 1). The net result was that neither the Na⁺ nor Ca²⁺ current densities were significantly affected by this treatment (see Fig. 4).

The enhanced Ca²⁺ current is dominated by L-type calcium channels

In vertebrate cells there are several different types of Ca²⁺ channels that can be distinguished pharmacologically and kinetically,

Figure 2. Calcium and sodium currents in treated glomus cells. *A*, The fast transient component of the inward current in this glomus cell grown in hypoxia (6% O₂) for 14 d was eliminated by the substitution of choline for Na⁺ in the bath perfusate. The prolonged inward current was abolished upon substitution of Co²⁺ (2 mM) for Ca²⁺ in the bath. *B*, Similarly, in this forskolin-treated glomus cell (13 d; 10 μM) the large transient current was blocked by choline substitution and the prolonged component blocked by the substitution of Co²⁺ (2 mM) for Ca²⁺. *C*, Current-voltage relation for isolated Ca²⁺ (choline substitution for Na⁺) and Na⁺ (Co²⁺ substituted for Ca²⁺) currents in a single glomus cell exposed to hypoxia (6% O₂) for 13 d. *D*, Steady-state inactivation curves for the Na⁺ and Ca²⁺ currents in a glomus cell treated with db-cAMP (1 mM; 8 d). In this cell, the steady-state Na⁺ inactivation curve (see Materials and Methods) was shifted ~ 25 mV to the left of the Ca²⁺ inactivation curve. The $V_{1/2}$ for the Na⁺ curve was -55 mV, while the $V_{1/2}$ for Ca²⁺ was -31 mV.

as well as by their voltage dependence (Nowicky et al., 1985; Bean, 1989; Hess, 1990). The predominant Ca²⁺ channel type present in neonatal and adult glomus cells is the dihydropyridine-sensitive L-type channel (Urena et al., 1989; Peers, 1990; Fieber and McCleskey, 1993; Buckler and Vaughan-Jones, 1994), one of at least three high-threshold Ca²⁺ channels (i.e., L, P, and N) with distinct pharmacology (Bean, 1989; Hess, 1990). We wondered whether long-term enhancement of the Ca²⁺ current in chronically hypoxic glomus cells was accompanied by a shift in expression of Ca²⁺ channel types, especially since N-type channel expression is known to be upregulated in related (neuroendocrine) PC12 cells by environmental factors (for references, see Hess, 1990). Table 1 indicates that the average value of $V_{1/2}$ for Ca²⁺ currents (determined from steady-state inactivation curves) was similar for control and treated cultures, i.e., ~ -35 mV, a value typical for L-type Ca²⁺ channels (McCleskey and Schroeder, 1991). To obtain a more quantitative description of the contribution of Ca²⁺ channel types to the enhanced Ca²⁺ current in chronically hypoxic cells, we tested the effects of L-type (nifedipine) and N-type (ω -conotoxin GVIA) blockers on the Ca²⁺ current. As shown in Figure 3, the Ca²⁺

current was considerably reduced by 10 μM nifedipine, but was relatively insensitive to 1 μM ω -conotoxin. The mean percent reduction ($\pm$ SE) in peak Ca²⁺ current amplitude by 10 μM nifedipine was $74 \pm 3.8\%$ ($n = 5$; range 59–80 %, for voltage step from -60 to -10 mV); in each of four experiments there was no detectable effect with 1 μM ω -conotoxin GVIA. The residual current after nifedipine in Figure 3 may be carried by dihydropyridine-insensitive L-type channels or some other unidentified Ca²⁺ channel type. In control (normoxic) glomus cells the inward Ca²⁺ current also appeared to be composed mainly of L-type calcium channels since a brief application of nifedipine (25 μM) reduced the whole-cell Ca²⁺ current by $71.0 \pm 8.8\%$ ($n = 5$). Thus, the properties of these Ca²⁺ currents in cultured normoxic and hypoxic glomus cells from neonatal rats appear similar to those in the adult, where 65% of the whole-cell Ca²⁺ current was inhibited by a similar concentration of nifedipine, and ω -conotoxin GVIA was likewise ineffective (Fieber and McCleskey, 1993). Interestingly, the magnitude of the calcium channel whole-cell currents in the latter study, where 110 mM Ba²⁺ was used to enhance these currents, was comparable to that observed in chronically hypoxic cells with only 2

Figure 3. The inward Ca^{2+} currents in hypoxia-treated glomus cells appear to be mainly composed of L-type Ca^{2+} channels. The isolated inward Ca^{2+} current in this glomus cell exposed to chronic hypoxia for 11 d was mainly ($\sim 79\%$) blocked by the L-type Ca^{2+} channel blocker nifedipine ($10 \mu\text{M}$) but was virtually unaffected by the N-type channel blocker ω -conotoxin GVIA ($1 \mu\text{M}$). Inward Na^{+} current was blocked by TTX (200 nM) and outward K^{+} currents by Cs^{+} and TEA (see Materials and Methods). Voltage step, -60 to -10 mV .

mM Ca^{2+} as current carrier (Table 1; Figs. 1, 2C, 3). This emphasizes the point that Ca^{2+} channel currents are substantially enhanced in glomus cells after chronic hypoxia.

Do hypoxia and cAMP specifically induce both Na^{+} and Ca^{2+} channels?

Measurements of input capacitance (Stea et al., 1992) and 3-D cell volume (Mills and Nurse, 1993; see also McGregor et al., 1984) indicate that glomus cells increase in size following chronic exposure to hypoxia or db-cAMP *in vitro*. This was also the case in the present experimental series and, in addition, treatment with forskolin was also effective in increasing input capacitance (see Table 1). These findings raise the question whether the augmented membrane currents described above are due

simply to the increase in cell size or to specific inductive mechanisms. To test this, we compared normalized Na^{+} and Ca^{2+} currents, elicited by steps to 0 mV from holding potentials of -60 and -100 mV , by dividing peak currents by the input capacitance and thereby obtained a measure of current density (Fig. 4). As shown in Figure 4A, the Na^{+} current density measured from both holding potentials was significantly higher in glomus cells exposed for 9–15 d to hypoxia ($2\text{--}3\times$ control) and db-cAMP ($4\text{--}5\times$ control), in agreement with our initial study (Stea et al., 1992). In addition, Figure 4A indicates that treatment with forskolin was as effective in inducing Na^{+} channels as db-cAMP, and that db-cGMP was ineffective. In marked contrast, however, none of the treatments significantly affected Ca^{2+} channel density estimated in the same way (Fig. 4B). Thus, it appears that the upregulation of Na^{+} , but not Ca^{2+} , currents in these cells by hypoxia and agents that increase intracellular cAMP are due to specific inductive mechanisms, unrelated to overall cell growth. Further evidence for this idea is given below.

Is protein synthesis required for Na^{+} channel induction by db-cAMP?

The selective increase in Na^{+} current density in glomus cells following exposure to chronic hypoxia and cAMP analogs could be due to synthesis of new Na^{+} channels, unmasking of silent ones already present in the plasma membrane, insertion of ones from a preexisting cytoplasmic pool, or combinations of these. We did not attempt to distinguish among these possibilities, but since the induction of Na^{+} channels by db-cAMP was rapid (2–3 d exposure, compared to 5–7 d for chronic hypoxia; see Stea et al., 1992), we asked whether protein synthesis was involved by using brief exposures to the protein synthesis inhibitor, cycloheximide ($90\text{--}180 \mu\text{M}$; Karst and Joels, 1991). To control for

Figure 4. A comparison of Na^{+} and Ca^{2+} current densities in chronically treated glomus cells. **A**, The Na^{+} current density in hypoxia ($6\% \text{ O}_2$), db-cAMP (1 mM), or forskolin ($10 \mu\text{M}$)-treated cells was significantly higher ($p < 0.01$) than control cells when measured from holding potentials of -60 mV , or -100 mV where Na^{+} channel inactivation is removed (see Fig. 2). Cells treated with $200 \mu\text{M}$ db-cGMP were not significantly different from controls. Densities were calculated on dividing peak currents elicited from voltage steps of -60 or -100 mV to 0 mV , by the whole-cell capacitance (see Materials and Methods). **B**, The Ca^{2+} current density calculated from cells in any of the treatments was not significantly different from controls; peak Ca^{2+} currents were recorded after 20 to 30 msec when Na^{+} currents were inactivated. Note: graphs are based on recordings from 81 cells and treatment duration was 8–15 d. Each bar represents the mean $\pm$ SE of a minimum of eight cells. * $p < 0.01$; Student's *t* test.

possible generalized toxic or deleterious effects of the drug, we compared the effects on Na⁺ current density with those on both K⁺ and Ca²⁺ current densities, using K⁺-containing and K⁺-free pipette solutions, respectively. In these experiments cycloheximide (90–160 μ M) was present only for the first 36–48 hr after the db-cAMP treatment began; thereafter, the cells were grown with db-cAMP alone for a further 1–2 d before recording. As shown with both recording conditions in Figure 5, *A* and *C*, the db-cAMP-induced increase in Na⁺ current density was abolished following cycloheximide treatment. In Figure 5*A*, Na⁺ currents were recorded along with K⁺ currents using K⁺-containing pipette solutions; however, in the same cells the K⁺ current density was not significantly affected by the drug (Fig. 5*B*). In Figure 5*C*, Na⁺ currents were recorded along with Ca²⁺ currents (K⁺-free pipette solutions), and here the Ca²⁺ current density was not significantly affected by the drug (Fig. 5*D*). Thus, cycloheximide does not appear to cause a nonspecific reduction in current densities, suggesting that its suppressive effect on the db-cAMP-augmented Na⁺ current density is selective, and likely due to a requirement for protein synthesis in the inductive process. We did, however, observe an effect of cycloheximide on cell size, reflected by $\sim 38\%$ reduction in whole-cell capacitance relative to age-matched controls (mean, 6.4 pF vs 4 pF, $n = 20$). This was probably mediated through inhibition of growth-related proteins. Since the capacitance is proportional to the square of the diameter, the effect is to decrease cell diameter by $\sim 20\%$.

Heterogeneity in augmented Na⁺ current response during hypoxia

In recordings from over 100 glomus cells grown under chronic hypoxia, a broad variability in responses, suggestive of a heterogeneous cell population, was observed (see SEM in Table 1; Stea et al., 1992). While only a few cells appeared not to respond at all to hypoxia, i.e., with neither an obvious increase in whole-cell capacitance nor Na⁺ current, a more substantial fraction showed an enlarged capacitance but with quite small peak inward currents < 90 pA typical of controls. Included among the latter was a single cell that appeared to be in the process of division and contained K⁺, but not Na⁺, active currents (not shown). This raises the possibility that cells undergoing DNA synthesis or mitosis may display an increase in cell size, but their immature status might be reflected in small or no inward Na⁺ currents, typical of neonatal rat glomus cells (Peers, 1990; Stea and Nurse, 1991). Doubtless the presence of such cells, whose proportion appears to increase during chronic hypoxia (Nurse and Vollmer, 1993), contributes to the heterogeneity in the peak Na⁺ current response. In addition, as suggested in the discussion, it is likely that different signal transduction pathways mediate cell hypertrophy and Na⁺ channel induction in these cells; hence, the heterogeneity may simply reflect the variability among cells in the activation of the respective pathways.

Action potentials in glomus cells after chronic treatments

The low density or absence of Na⁺ channels in glomus cells of both neonatal and adult rats indicates that under normal environmental pO₂ they rarely, if at all, fire Na⁺ action potentials (Stea and Nurse, 1991; Fieber and McCleskey, 1993; see also Peers, 1990). The increase in number and density of Na⁺ channels after chronic hypoxia, together with the augmented Ca²⁺ currents (Figs. 1, 2) and tendency for the overall K⁺ current density to decline (Stea et al., 1992), point to an increase in electrical excitability. We confirmed this in cultures grown in

chronic hypoxia or db-cAMP by first identifying cells with substantial peak Na⁺ currents (> 150 pA) under voltage clamp, and then switching to current clamp to test their responses to depolarizing stimuli. As shown in Figure 6*A–D*, this “hypoxic” glomus cell had an enlarged peak Na⁺ current of ~ 300 pA for a voltage step from -60 to 0 mV (Fig. 6*D*). On switching to current clamp, the resting or zero-current potential was ~ -55 mV; following a 200 msec suprathreshold depolarizing pulse, the cell gave two active responses, in which the second was of longer duration reminiscent of Ca²⁺ spikes (Fig. 6*A*). The second response was blocked reversibly with 2 mM cobalt (Fig. 6*B,C*), confirming a significant Ca²⁺ component and, at the same time the initial spike was reduced in amplitude and was of longer duration (Fig. 6*B*). The prolongation of the initial spike in Figure 6*B* was presumably due to blockade of Ca²⁺ entry, thus preventing activation of Ca²⁺-activated K⁺ channels known to be present in these cells (Stea et al., 1991; see also, Peers, 1990). The initial spike in Figure 6*A* was also considerably suppressed by substituting choline for extracellular Na⁺ (not shown), and in other examples by choline substitution or TTX ($n = 4$). The cell in Figure 6*A–D* also displayed occasional spontaneous spike waveforms (not shown), similar to Figure 6, *A* and *C*, consistent with an increased excitability. It therefore appears that both Na⁺ and Ca²⁺ ions contribute to the spike activity in chronically hypoxic rat type 1 cells, and this also appears to be the case for cells grown in db-cAMP, as illustrated in Figure 6, *E* and *F*.

Discussion

In this study we provide evidence for long-term modulation of membrane currents and electrical excitability in rat carotid body chemoreceptors (glomus or type 1 cells), following chronic exposure to hypoxia. Since the modifications occurred in isolated type 1 cell clusters, growing in controlled gaseous, fluid, and cellular environments, it appears that effects are a direct consequence of chronic receptor stimulation by low pO₂, and do not require secondary cardiovascular factors associated with *in vivo* exposure to hypoxia. We interpret the augmented inward sodium and calcium currents, and resulting increased electrical excitability in type 1 cells, as part of a cascade of adaptive cellular responses that are likely to be physiologically important during the period of ventilatory acclimatization to hypoxia, when carotid body chemosensitivity is enhanced (Barnard et al., 1987; Nielsen et al., 1988). Our data are also consistent with the idea that cAMP-mediated signal transduction pathways, that are activated during acute hypoxia (Perez-Garcia et al., 1990; Delpiano and Acker, 1991; Wang et al., 1991), are also involved in the long-term ion channel regulation in type 1 cells.

Physiological significance of Na⁺ channel induction

Perhaps the most interesting physiological effect of chronic hypoxia on isolated rat carotid body chemoreceptors is the induction of Na⁺ channels. It is now generally accepted that in both adult and neonatal rat type 1 cells, voltage-gated Na⁺ channels are either absent (Peers, 1990; Stea and Nurse, 1991; Fieber and McCleskey, 1993), or present at very low density (Stea and Nurse, 1991). The general conclusion from these studies is that rat type 1 cells do not or rarely generate Na⁺ action potentials and are spontaneously inactive, unlike their adult rabbit counterparts (see, Urena et al., 1989; Biscoe and Duchon, 1990; Gonzalez et al., 1992); moreover, the lack of this Na⁺ spike mechanism in the rat is not developmentally related (Stea and Nurse, 1991; Fieber and McCleskey, 1993). Thus, the increased density

Figure 5. The effect of inhibition of protein synthesis on Na^+ , K^+ , and Ca^{2+} current densities in glomus cells treated with db-cAMP. **A**, The Na^+ current density is significantly increased in db-cAMP-treated cells (3–4 d; 1 mM) during recordings with a K^+ -containing pipette solution. This increase is blocked by the addition of 180 μM cycloheximide for the first 36–48 hr under the same conditions. **B**, The K^+ current density is slightly lower in db-cAMP-treated glomus cells (3–4 d; 1 mM) in agreement with our previous study (Stea et al., 1992), while in cells treated with db-cAMP plus cycloheximide (2 d), it was slightly, although not significantly, higher. **C**, In these experiments, where recordings were performed with K^+ -free pipette solutions, the Na^+ current density is similarly increased significantly in db-cAMP treated cells (3–4 d; 1 mM). This increase is again blocked by the addition of 90 μM cycloheximide for the first 36–48 hr under the same conditions. **D**, The Ca^{2+} current densities measured in cells treated with db-cAMP and db-cAMP + cycloheximide (see **C**) are not significantly different from controls. Note: Na^+ and Ca^{2+} current densities were calculated from peak currents elicited by voltage steps of -60 to 0 mV; K^+ current density was calculated from outward currents elicited by a voltage step of -60 to 50 mV (see Materials and Methods). * $p < 0.01$; Student's t test.

of Na^+ channels, which develops gradually over 5–12 d following chronic hypoxia (6% O_2) *in vitro* (Stea et al., 1992), appears to be a specific adaptive response resulting in increased excitability of type 1 cells in the rat. In contrast, normal adult rabbit type 1 cells generate spontaneous Na^+ -dependent action potentials whose frequency is modulated by acute changes in pO_2 , via closure of O_2 -sensitive K^+ channels (Urena et al., 1989; Gonzalez et al., 1992; Lopez-Barneo et al., 1993). Since these K^+ channels are activated at depolarized voltages more positive than -30 mV in both species, it remained unclear in the rat how they are affected by acute hypoxia, given that the cells appear to be spontaneously inactive at resting potentials negative to -30 mV (see Gonzalez et al., 1992; Fieber and McCleskey, 1993; Buckler and Vaughan-Jones, 1994). There is recent evidence, however,

that neonatal rat type 1 cells depolarize during acute hypoxia and generate presumptive Ca^{2+} action potentials when recording conditions are more physiological, i.e., at 37°C and in the presence of bicarbonate/ CO_2 buffer (Buckler and Vaughan-Jones, 1994). Thus, the induction of the Na^+ spike mechanism in rat type 1 cells, rendered chronically hypoxic, may have several consequences including (1) an increased probability for spontaneous firing (as occurred in one case during our study at room temperature) due to a lower firing threshold; (2) an extension of the range of firing frequencies of which the receptor is capable, due to shortening of the action potential duration; and (3) an opportunity for the cell to reach more positive membrane potentials, and, thus, facilitate calcium entry and neurotransmitter release. The increase in Na^+ channel density may well be one

Figure 6. Active responses in glomus cells treated chronically with hypoxia or db-cAMP. *A*, A glomus cell exposed to hypoxia (6% O₂) for 8 d produced two active responses with different time courses after a suprathreshold depolarizing pulse. The resting potential in this cell was -55 mV. *B*, Perfusion with the same bathing solution with 2 mM Co²⁺ eliminated the second active response while prolonging the first. *C*, Washout of the Co²⁺-containing bath solution caused a recovery of the two active responses, although the second was attenuated. *D*, Voltage-clamp recording in normal K⁺-containing solutions from the same cell as *A–C* shows a large inward current of ~ 300 pA; voltage step, -60 to 0 mV. *E*, Voltage-clamp recording from a db-cAMP-treated cell (200 μ M; 7 d) shows a similar large inward Na⁺ current. *F*, Current-clamp recording from the same db-cAMP-treated cell as in *E* shows several active responses that appear to contain both fast Na⁺ and slower Ca²⁺ components.

adaptive mechanism type 1 cells use to increase receptor sensitivity during acclimatization to chronic hypoxia. Among sensory receptors, modulation of impulse frequency appears to be a general mechanism that allows for greater sensitivity than would be feasible in a quiescent cell (see Eckert et al., 1988), which appears to be the physiological state of *rat* type 1 cells under normoxic conditions (Fieber and McClesky, 1993; Buckler and Vaughan-Jones, 1994). Following depolarization however, we found that several “chronically hypoxic” type 1 cells with enhanced Na⁺ currents could generate multiple spikes that appeared to contain both short-duration Na⁺ and longer-duration Ca²⁺ components, a feature generally not present in normoxic cells. It remains to be determined, however, whether *in vivo* exposure of rats to chronic hypoxia results in type 1 cells acquiring the potential for Na⁺ spiking, and that our findings are not an artefact of the *in vitro* conditions. We consider the latter possibility unlikely, and, in fact, it appears that our results may

have broad applicability even to the CNS, where Na⁺ channels have recently been reported to increase in number in newborn rat brain after *in utero* hypoxia (Xia and Haddad, 1994).

Contrasting mechanisms for augmented Na⁺ and Ca²⁺ currents during chronic hypoxia

In type 1 cells both macroscopic inward Na⁺ and Ca²⁺ currents were significantly enlarged after 9–14 d exposure to chronic hypoxia. Since under these conditions the cells also undergo hypertrophy, as indicated by a two- to fourfold increase in whole-cell capacitance (Table 1; see also Stea et al., 1992) and 3-D cell volume (Mills and Nurse, 1993), it was of interest to compare the normalized current densities. We found that whereas the Na⁺ current density was significantly enhanced following chronic hypoxia, the Ca²⁺ current density was similar to control (normoxia). These findings, together with the observations that Na⁺ currents are frequently absent from control cells, and that

the increased Na^+ current density in dibutyl cAMP-treated cultures required protein synthesis (since it was selectively abolished by the protein synthesis inhibitor, cycloheximide) suggest that specific inductive mechanisms are responsible for the expression of functional Na^+ channels in these type 1 cells. One likely explanation is that there was an upregulation of Na^+ channel gene expression and *de novo* synthesis of Na^+ channel proteins during the period of chronic hypoxia. It is noteworthy that in the present study elevators of intracellular cAMP (db-cAMP and forskolin) mimicked this effect of chronic hypoxia, and in rat skeletal muscle cells cAMP increases both the number of Na^+ channels as well as an 8.5 kb mRNA encoding the Na^+ channel α subunit (Offord and Catterall, 1989). Alternative explanations for the enhanced Na^+ current include unmasking of preexisting but nonfunctional channels in the plasma membrane, or recruitment of channels from a dormant intracellular pool. In contrast, given that the cells normally produce L-type Ca^{2+} channels (Peers, 1990; Fieber and McCleskey, 1993; Buckler and Vaughan-Jones, 1994), it would appear that the augmented Ca^{2+} current seen during chronic hypoxia was not due to a *specific* inductive process since the current density was unaltered; rather, the simplest explanation is that functional Ca^{2+} channels were incorporated into the plasma membrane *pari passu* with the cell growth that occurred during chronic hypoxia. These channels could be synthesized along with other membrane proteins during the period of cell growth, or recruited to plasma membrane by insertion from an intracellular store (see Passafaro et al., 1994). As discussed above for Na^+ channels, our data do not rule out alternative explanations; for example, the channels may already exist in the plasma membrane in a cryptic or non-functional state, and then become functional during chronic hypoxia, for example, by protein phosphorylation. Such a mechanism has been proposed for the beta-adrenergic modulation of calcium currents via cAMP-dependent protein kinase in heart cells, though this effect is rapid, occurring over a time scale of seconds (see Bean, 1989; Hess, 1990). Regardless of the mechanism, it appears that the enhanced Ca^{2+} current seen after chronic hypoxia was dominated by L-type Ca^{2+} channels as in normal type 1 cells, since it was markedly inhibited by the dihydropyridine blocker, nifedipine, but was unaffected by the N-type channel blocker, ω -conotoxin GVIA.

It is also possible that our method of estimating Ca^{2+} channel density may not accurately reflect the true physiological effect of the enhanced calcium currents on type 1 cells. This estimate assumes a uniform distribution of channels on the cell surface, but there is now compelling evidence from a variety of preparations that calcium channels are strategically clustered or concentrated in local hot spots at neurotransmitter release sites (Lipscombe et al., 1988; Robitaille et al., 1993). Hence, if the calcium channels that appear in type 1 cells after chronic hypoxia are directed towards strategic domains within the plasma membrane, then local increases in channel density may occur but will not be resolved by our technique. Such local increases are potential mechanisms for increasing receptor sensitivity during acclimatization to chronic hypoxia by facilitating neurotransmitter release.

Cyclic AMP as an intracellular mediator of the effects of chronic hypoxia

Our data implicate cAMP as a potential intracellular mediator of at least some of the effects of chronic hypoxia on type 1 cells. Interest in cAMP arose from previous studies indicating that

acute hypoxia causes a dose-dependent increase in cAMP levels in the carotid body (Perez-Garcia et al., 1990; Delpiano and Acker, 1991), and particularly in type 1 cells (Wang et al., 1991). These findings, together with the well-documented ability of intracellular cAMP to promote both short- and long-term modulation of ion channels in a variety of systems (Barres et al., 1989; Offord and Catterall, 1989; Hess, 1990; Kalman et al., 1990; Dolphin, 1992), led us to consider this second messenger as a potential mediator of the effects of chronic hypoxia. Consistent with this idea, we found that agents that cause cAMP elevation, i.e., dibutyl cAMP and forskolin, enhanced both Na^+ and Ca^{2+} currents as well as whole-cell capacitance (or cell size) in cultured type 1 cells grown under control (normoxic) conditions for 9–14 d. All three effects, which could not be mimicked by elevation of a different cyclic nucleotide, i.e., cyclic GMP, are qualitatively similar to those obtained with chronic hypoxia. However, on comparing the effects of the various treatments on the properties of type 1 cells (Table 1; see also Stea et al., 1992), it is apparent that hypoxia (6% O_2) had the most pronounced effect on increasing cell size and peak Ca^{2+} current; in contrast, the elevators of cAMP were more effective in increasing peak Na^+ current. In light of these results, we propose that cAMP is the likely mediator of the inductive effect of chronic hypoxia on the Na^+ current, though other signals, for example, hypoxia-evoked rise in intracellular Ca^{2+} (Biscoe and Duchon, 1990; Buckler and Vaughan-Jones, 1994), may be more important for cell hypertrophy and the concomitant increase in Ca^{2+} current. We are currently testing this hypothesis by growing cells in the presence of Ca^{2+} channel blockers. Whatever the explanation, it is clear that isolated type 1 cells display a novel morphological and physiological plasticity, resulting in cell growth and modulated ion channel expression, when exposed chronically to low pO_2 environments.

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