Oxygen Sensing by Ion Channels and Chemotransduction in Single Glomus Cells

R. J. MONTORO, J. UREÑA, R. FERNÁNDEZ-CHACÓN, G. ALVAREZ DE TOLEDO, and J. LÓPEZ-BARNEO

From the Departamento de Fisiología Médica y Biofísica, Facultad de Medicina, Universidad de Sevilla, E-41009 Sevilla, Spain

ABSTRACT We have monitored cytosolic [Ca^{2+}] and dopamine release in intact fura-2–loaded glomus cells with microfluorimetry and a polarized carbon fiber electrode. Exposure to low Po_2 produced a rise of cytosolic [Ca^{2+}] with two distinguishable phases: an initial period (with Po_2 values between 150 and ~70 mm Hg) during which the increase of [Ca^{2+}] is very small and never exceeds 150–200 nM, and a second phase (with Po_2 below ~70 mm Hg) characterized by a sharp rise of cytosolic [Ca^{2+}]. Secretion occurs once cytosolic [Ca^{2+}] reaches a threshold value of 180 ± 43 nM. The results demonstrate a characteristic relationship between Po_2 and transmitter secretion at the cellular level that is comparable with the relation described for the input (O_2 tension)–output (afferent neural discharges) variables in the carotid body. Thus, the properties of single glomus cells can explain the sensory functions of the entire organ. In whole-cell, patch-clamped cells, we have found that in addition to O_2-sensitive K^+ channels, there are Ca^{2+} channels whose activity is also regulated by Po_2. Ca^{2+} channel activity is inhibited by hypoxia, although in a strongly voltage-dependent manner. The average hypoxic inhibition of the calcium current is 30% ± 10% at −20 mV but only 2% ± 2% at +30 mV. The differential inhibition of K^+ and Ca^{2+} channels by hypoxia helps to explain why the secretory response of the cells is displaced toward Po_2 values (below ~70 mm Hg) within the range of those normally existing in arterial blood. These data provide a conceptual framework for understanding the cellular mechanisms of O_2 chemotransduction in the carotid body.

INTRODUCTION

The carotid bodies have been recognized for decades as sensory organs capable of transducing reductions in arterial O_2 tension (Po_2) into afferent nerve signals conveying the information to the brain stem respiratory centers to evoke hyperventilation. Nevertheless, the cellular and molecular mechanisms underlying chemotransduction have remained obscure. Among the various cellular elements in the carotid body, glomus cells have been considered as key elements in the sensory process, since their integrity is essential for the normal hypoxia sensitivity of the organ (Verna et al., 1975), they contain numerous cytosolic granules rich in catecholamines and other putative transmitters, and they establish well-defined synapses with afferent nerve terminals (for reviews see McDonald, 1981; Fidone and González, 1986). However, direct evidence for their sensory role has come from recent patch-clamp experiments performed on enzymatically dispersed cells. It has been shown that glomus cells, which are of neuroectodermal origin, can generate Na^+- and Ca^{2+}-dependent action potentials repetitively and that their special chemoreceptive properties are based upon the presence of O_2-sensitive K^+ channels whose open probability is decreased by low Po_2 (López-Barneo et al., 1988; López-López et al., 1989; Delpiano and Hescheler, 1989; Peers, 1990; Stea and Nurse, 1991; Ganfornina and López-Barneo, 1991, 1992*a*). Since hypoxia increases electrical excitability (López-López et al., 1989) and dopamine release (Fishman et al., 1985) in cultured glomus cells, it has been proposed that they may function as O_2-sensitive presynaptic-like elements transducing reductions in arterial Po_2 into an external calcium-dependent secretory signal (for reviews see González et al., 1992; López-Barneo et al., 1993). This scheme of chemotransduction has received strong support from recent work conducted in several laboratories showing that the increase of cytosolic [Ca^{2+}] in response to low Po_2 depends on Ca^{2+} entry through volt-

Address correspondence to Dr. José López-Barneo, Departamento de Fisiología Médica y Biofísica, Facultad de Medicina, Avenida Sánchez Pizjuán, 4, E-41009 Sevilla, Spain.

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age-gated channels (Sato et al., 1991; Buckler and Vaughan-Jones, 1994; Ureña et al., 1994). Furthermore, the coupling between calcium entry and quantal dopamine secretion in single glomus cells has been directly demonstrated (Ureña et al., 1994).

Despite the considerable amount of experimental data stressing the importance of O_2-sensitive K^+ channels in carotid body function, it is still uncertain whether the responses to hypoxia of individual glomus cells can account for the properties of the entire organ. In fact, the most serious criticism to the membrane ion channel model of chemotransduction is motivated by an apparent discrepancy existing between the Po_2 values at which dispersed glomus cells and the entire carotid body are responsive to hypoxia (see, for example, Biscoe and Duchen, 1990; Lahiri, 1994). The inhibition of O_2-sensitive K^+ channel activity in dispersed cells is produced when Po_2 decreases to <150 mm Hg (López-López et al., 1989; Ganfornina and López-Barneo, 1991), whereas the acceleration of the discharge rate of individual sinus nerve afferent fibers recorded in vivo is observed only after blood Po_2 reaches values $<\sim$70 mm Hg (Horbein, 1968; Biscoe et al., 1970). Therefore, it is of major importance to establish to what extent the basic features of chemotransduction can be explained by the properties of individual glomus cells or, on the contrary, whether the input (O_2-sensing)–output (afferent neural discharges) relationships of the carotid body are determined by other variables. These basic questions have become experimentally addressable because of recent technical developments that have allowed us to study in individual glomus cells the relations existing between either membrane ionic conductances, cytosolic [Ca^{2+}], or dopamine release with Po_2 in the surrounding extracellular milieu. We report here that the relationship between Po_2 and transmitter secretion at the cellular level is comparable with that described for the input–output variables in the carotid body. Thus, the properties of single glomus cells explain the chemoreceptive function of the entire organ. We have found O_2-sensitive Ca^{2+} channels in glomus cells whose activity is inhibited by low Po_2 in a strongly voltage-dependent manner. The combined differential inhibition of K^+ and Ca^{2+} channels by hypoxia helps to explain why the secretory response of the cells is displaced toward Po_2 values similar to those normally existing in arterial blood. These results provide a comprehensive view of the cellular mechanisms of chemotransduction.

METHODS

Cell Preparation and Electrophysiologic Recording

Experiments were performed on glomus cells isolated from rabbit carotid bodies. The procedures followed for enzymatic cell dispersion and culture were the same as described previously (López-Barneo et al., 1988; Ureña et al., 1989). Cells were plated on slivers of glass coverslips treated with poly(L-lysine) and used for recording between 12 h and 3 d after dissociation. During the experiments a coverslip was placed in a chamber of ~0.2 ml with a continuous flow of solution that could be replaced in 15–20 s. The bath solution was equilibrated with air (Po_2 ~150 mm Hg) or mixtures of N_2 and air to obtain the desired O_2 concentrations. Po_2 in the chamber was monitored with an O_2-sensing electrode (Ganfornina and López-Barneo, 1992*a*). Macroscopic calcium and potassium currents were recorded using the whole-cell configuration of the patch-clamp technique (Hamill et al., 1981). The details of the methods followed in our laboratory are given elsewhere (Ureña et al., 1989). Solution compositions and other experimental variables are given in the figure legends.

Monitoring of Cytosolic [Ca^{2+}]

Cytosolic [Ca^{2+}] was estimated in unclamped cells loaded with fura-2 by incubation for 30 min at 37°C with saline containing 1 μM fura-2 acetoxymethyl ester. The external solution contained (in millimolars): 140 NaCl, 2.7 KCl, 2.5 $CaCl_2$, 1 $MgCl_2$, 5 glucose, 10 Hepes, pH 7.3. In the high external K^+ solutions, NaCl was replaced equimolarly for KCl. Experiments were performed on an inverted microscope with standard optical components and equipped for epifluorescence and photometry (Almers and Neher, 1985). For the two excitation wavelengths, we used the filters short-wave pass SWP 357 (excitation at ~360 nm) and band pass BP 380 (excitation at 380 nm and 10 nm bandwidth). Fluorescence from the cells was measured by a dual wavelength photometer. The two output voltage signals from the photometer were digitized and displayed on line on the screen of a computer in parallel with the estimated [Ca^{2+}] (Toledo-Aral et al., 1993). Calibration of the fluorescence signals in terms of [Ca^{2+}] was performed in vitro as described elsewhere (Grynkiewicz et al., 1985).

Electrochemical Detection of Dopamine Release

Dopamine secretion was monitored in amperometric mode (Wightman et al., 1991) with a glass-sealed 8-μm-diameter carbon electrode fabricated following a procedure previously described (Chow et al., 1992; Alvarez de Toledo et al., 1993) We held the carbon fiber at a constant voltage of +650 mV, a potential more positive than the oxidation potential of dopamine, and thus the amperometric signal mostly represents dopamine secretion. Cyclic voltammograms, obtained by application of voltage ramps from −600 to +1,000 mV at a rate of 170 V/s, were characteristic of dopamine, with the typical reduction (at ~−400 mV) and oxidation (at ~+800 mV) peaks (Ureña et al., 1994). The three signals recorded in parallel (fluorescence, amperometry, and Po_2 values) were stored on tape and analyzed off line. Experiments were conducted at room temperature (22–25°C).

RESULTS

Secretory Response to Hypoxia in Single Glomus Cells

Glomus cells are able to release dopamine on exposure to low Po_2 because of Ca^{2+} influx through voltage-dependent channels. This secretory response to hypoxia is illustrated in Fig. 1 with recordings from an isolated,

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fura-2–loaded cell in which we simultaneously monitored dopamine release with a polarized micro-carbon fiber and the changes in cytosolic [Ca^{2+}] occurring in parallel with the modifications of environmental oxygen tension. The secretory activity appears as spikelike current transients, each one representing a package of dopamine released from single secretory vesicles (see Wightman et al., 1991; Chow et al., 1992; Ureña et al., 1994). To facilitate comparison among the different variables, we also include in the figure the time integral of the discrete secretory events, which gives in terms of charge the cumulative secretory activity over the entire period of exposure to low Po_2. These recordings indicate that during the slow switching from normoxia (Po_2 = 150 mm Hg) to hypoxia ($Po_2 \sim 20$ mm Hg) there is an increase in cytosolic [Ca^{2+}] that triggers dopamine release. In most glomus cells, the response to hypoxia appeared to have two distinguishable phases. As Po_2 begins to decrease below 150 mm Hg, a small, although maintained, rise of cytosolic [Ca^{2+}] that evokes little secretory activity is observed. Secretory events appearing randomly during this initial period of the hypoxic exposure were likely a result of spontaneous release, since they were also detected under normoxic conditions. Once Po_2 reaches values near ~50 mm Hg (*vertical discontinuous line* in Fig. 1), there is an abrupt increase in internal Ca^{2+} and in parallel a marked acceleration of vesicle fusion and dopamine release. Recovery of the basal [Ca^{2+}] and arrest of secretion were produced after reintroduction of the normoxic solution to the chamber. Therefore, although the transition from normoxia to severe hypoxia can lead to an almost immediate rise of cytosolic [Ca^{2+}], dopamine release is delayed until the internal [Ca^{2+}] reaches a value of ~150–200 nM. The cytosolic [Ca^{2+}] necessary to trigger secretion was estimated in experiments in which Ca^{2+} influx evoked by membrane depolarization with high extracellular K^+ produced a slow rise in [Ca^{2+}], which allowed a more precise measurement of the cytosolic [Ca^{2+}] coincident with the onset of secretion (Fig. 2). In our experimental conditions the estimated mean resting cytosolic [Ca^{2+}] was ~60 nM and the apparent [Ca^{2+}] threshold required for the initiation of a clear episode of dopamine release was 180 ± 43 nM (mean ± SD, n = 5 cells treated with 30 mM external K^+). Exposure to external K^+ concentrations (10 or 15 mM) that produced a maintained elevation of internal [Ca^{2+}] below threshold did not elicit secretion (data not shown). These results indicate that glomus cells have a distinctive response to low Po_2. The rise of cytosolic [Ca^{2+}] over a threshold level seems to be a prerequisite for secretion; thus, moderate reductions of O_2 tension that induce only small subthreshold elevations of cytosolic [Ca^{2+}] do not elicit dopamine release. However, lower Po_2 values can evoke a robust secretory response, triggered by a typical sharp, suprathreshold rise of [Ca^{2+}] inside the glomus cell.

The changes in the cytosolic [Ca^{2+}] of glomus cells in response to hypoxia are further illustrated in Fig. 3 with data obtained following an experimental protocol that consisted of the application of several pulses of Po_2 <150 mm Hg. These recordings again show the characteristic response to hypoxia described above, which appeared in different experiments with quantitative varia-

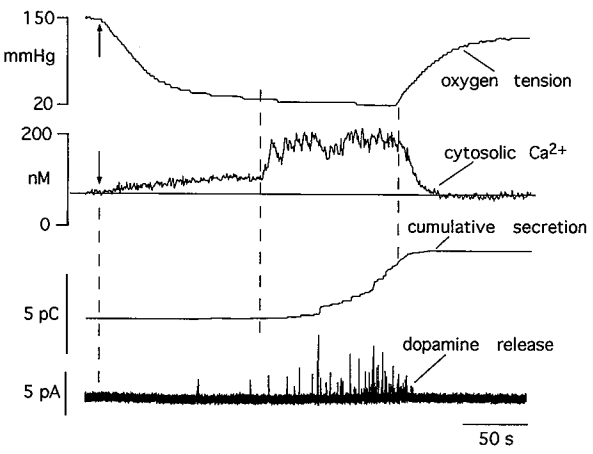

FIGURE 1. Secretory response of single glomus cells to low Po_2. Parallel changes of O_2 tension, cytosolic [Ca^{2+}], and dopamine secretion in a glomus cell in response to hypoxia. The cumulative secretion signal (in picoCoulombs) is the time integral of dopamine release. The maximal cumulative secretion value used for the calculation of the plot in Fig. 4 *B* is indicated by the vertical dashed line. Other vertical lines are drawn to facilitate the comparison of the changes of the different variables with time. The standard external solution contained (in millimolars): 140 NaCl, 2.7 KCl, 2.5 $CaCl_2$, 1 $MgCl_2$, 5 glucose, 10 HEPES, pH 7.3.

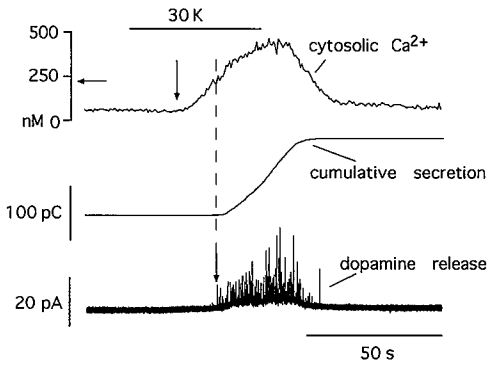

FIGURE 2. Secretory response of a glomus cell to high extracellular K^+. Simultaneous recording of cytosolic [Ca^{2+}] and dopamine secretion during the exposure to high extracellular K^+. In this experiment the fast rate of secretion results in the partial fusion of the individual spikes into a broad concentration envelope. The cumulative secretion signal (in picoCoulombs) is the time integral of dopamine release. Recording solution as in Fig. 1 with NaCl replaced equimolarly for KCl. The application of the high K^+ solution is indicated by the horizontal bar.

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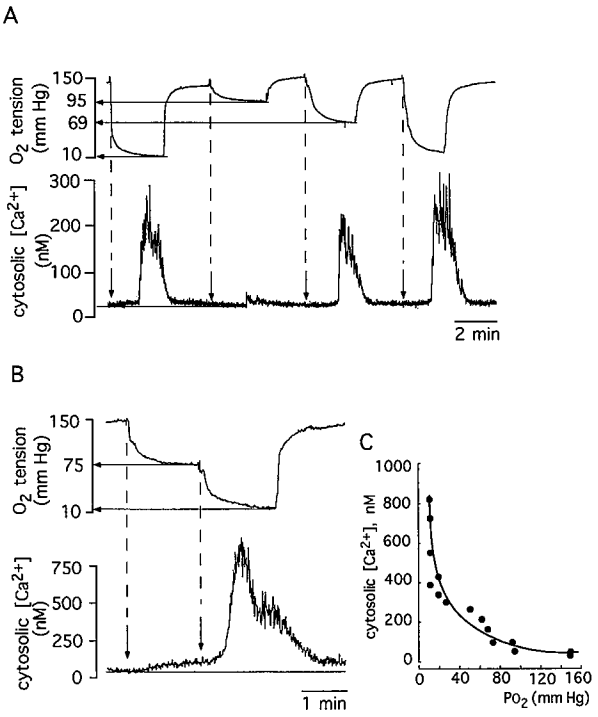

FIGURE 3. (*A* and *B*) Modifications of cytosolic $[Ca^{2+}]$ in dispersed glomus cells during exposure to external solutions equilibrated with various O_2 tensions. Solutions and other experimental variables as in Fig. 1. (*C*) Plot of cytosolic $[Ca^{2+}]$ reached after the application of external solutions equilibrated with various levels of Po_2. Values are from four cells subjected to the experimental protocol indicated in *A*. The line over the data points has been drawn by eye.

tions. Fig. 3 *A* demonstrates that, as shown in Fig. 1 with the ramplike hypoxic exposures, large changes of cytosolic Ca^{2+} are only observable with Po_2 levels of $\leqslant\sim$70 mm Hg, and that below this Po_2 value, the rise of internal $[Ca^{2+}]$ depends on the severity of hypoxia. A similar result is illustrated in Fig. 3 *B* with recordings from a cell in which, despite the protracted exposure to a Po_2 of 75 mm Hg, cytosolic $[Ca^{2+}]$ only increased up to $\sim$150 nM. The abrupt suprathreshold increase of $[Ca^{2+}]$ (up to near 1 μM) was produced only after a further decrease of Po_2. Thus, the degree of hypoxia drastically determines the pattern of response in glomus cells.

Relationship between O_2 Tension, Cytosolic $[Ca^{2+}]$, and Dopamine Secretion

Since Ca^{2+} entry and secretion are fast processes occurring in the range of milliseconds (cf. Burgoyne and Morgan, 1995), the slow ramplike exposures to hypoxia were used as an approximation to study the input–output relations in single glomus cells. The relationships between O_2 tension and either cytosolic $[Ca^{2+}]$ or dopamine secretion are shown, respectively, in Fig. 4, *A* and *B*, with data pooled from several (n = 4) experiments similar to the one described in Fig. 1. The hyperbole-like Po_2–$[Ca^{2+}]$ relationship (Fig. 4 *A*) is comparable with the one obtained with a different protocol consisting of the application of steady levels of Po_2 (see *inset C* of Fig. 3). A similar shape, although displaced toward lower Po_2 values, has the Po_2–dopamine release relation of single glomus cells (Fig. 4 *B*). This graph clearly demonstrates that although virtually no secretory activity was apparent at a Po_2 between 150 and 70 mm Hg, it abruptly increased once the cytosolic $[Ca^{2+}]$ reached suprathreshold values. As shown in Fig. 1, low Po_2 produces a progressive increase in the number of quantal events (rate of vesicle fusion) and in the slope of the cumulative secretory signal (rate of dopamine release). In Fig. 4 *B* we plot cumulative secretion because we assume that during a ramplike exposure to hypoxia, this is the variable that most closely reproduces the accumulation of transmitter in the glomus cell–afferent fiber synapse, which determines the firing frequency in the sinus nerve. We are aware that the steepness of the Po_2–dopamine release curve below 70 mm Hg is surely accentuated because we have not taken into consideration diffusion or reuptake of the transmitter released. However, it is worth stressing the remarkable similarity of plot 4 *B* with the O_2 dependence of the afferent nerve fiber activity in the entire carotid body (Horbein, 1968; Biscoe et al., 1970). These results indicate that the input–output relations of single glomus cells can

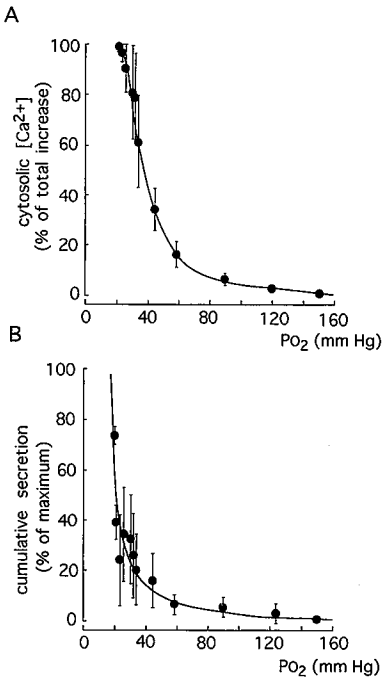

FIGURE 4. Changes in cytosolic $[Ca^{2+}]$ (*A*) and dopamine secretion (*B*) as a function of environmental O_2 tension (Po_2). Values in the ordinate are normalized and represent measurements (mean ± SD, n = 4) from experiments similar to the one illustrated in Fig. 1. The lines over the data points have been drawn by eye.

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explain the major physiologic features of the entire carotid body.

Hypoxic Inhibition of K^+ Channel Activity and the Changes of Action Potential Firing Frequency in Glomus Cells

Inhibition of O_2-sensitive K^+ channels in response to low Po_2 is presumed to be the initial step in the sequence of events leading to the rise of cytosolic Ca^{2+} and dopamine secretion in glomus cells (see López-Barneo et al., 1993). This hypothesis has recently received strong support from experiments demonstrating that the increase of cytosolic Ca^{2+} on exposure to hypoxia depends on Ca^{2+} entry through voltage-dependent Ca^{2+} channels (Buckler and Vaughan-Jones, 1994; Ureña et al., 1994). In rabbit glomus cells, the rise of cytosolic Ca^{2+} in response to hypoxia is abolished by either removal of external Ca^{2+} or the application of inorganic Ca^{2+} channel blockers (Ureña et al., 1994). However, there are important features of this conceptual scheme not yet studied in detail. For example, the relationship between Po_2 and K^+ channel activity is not well understood, since previous work in our laboratory documenting the dose-dependent hypoxic inhibition of the K^+ current (López-López et al., 1989) was done without direct monitoring of the actual O_2 tension in the recording chamber. We have now measured the K^+ channel activity in glomus cells subjected to patch-clamp and exposed to ramplike reductions of Po_2 following the same experimental protocol of Fig. 1, thereby allowing us to compare the O_2 dependence of the various steps in the process of chemotransduction.

The macroscopic, voltage-dependent Ca^{2+} and K^+ currents generated in a glomus cell during 20-ms depolarizations to +20 mV are shown in Fig. 5 *A*. During exposure to hypoxia the peak outward current, representing K^+ channel activity, was reversibly inhibited by ~25%, whereas the inward current and the tail, both caused by the activity of Ca^{2+} channels (Ureña et al., 1989), were practically unaltered. The modification of the peak outward current during the hypoxic run is shown in Fig. 5 *B*, where it can be clearly appreciated that the reduction of K^+ channel activity closely follows the decrease of Po_2. A quantitative summary of these experiments is given in Fig. 5 *C*. In addition to the average K^+ channel activity–Po_2 relationship (n = 8 cells), we have plotted in normalized ordinates the cytosolic $[Ca^{2+}]$- and dopamine release-Po_2 curves of Fig. 4 to facilitate the comparison of the various O_2-dependent variables. Note that whereas large changes of cytosolic $[Ca^{2+}]$ and secretion are only observed below Po_2 ~ 70 mm Hg, the hypoxic inhibition of K^+ channel activity is roughly linear in the Po_2 range studied and therefore detectable when O_2 tension decreases to just below normoxic values (150 mm Hg).

Because dispersed rabbit glomus cells are able to generate large Na^+- and Ca^{2+}-dependent action potentials repetitively (Duchen et al., 1988; Ureña et al., 1989), it can be expected that the inhibition of K^+ channel activity by low Po_2 would produce an increase in the firing frequency of the cells (López-Barneo et al., 1988). In fact, we have previously shown in whole-cell patch-clamped cells that the electrical excitability, tested as the action potential firing frequency in the ini-

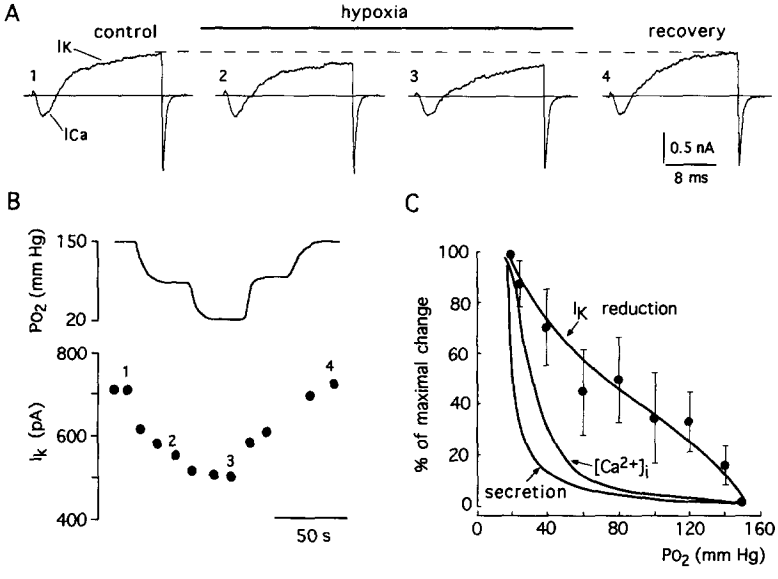

FIGURE 5. Dose-dependent inhibition of K^+ channel activity by low Po_2. (*A*) Macroscopic calcium and potassium currents recorded from a dispersed glomus cell during 20-ms step depolarizations to +20 mV from a holding potential of −80 mV. Exposure to hypoxia (switching from an external solution equilibrated with a Po_2 ~ 150 mm Hg to another with a Po_2 ~ 20 mm Hg). Records 2 and 3 induce a reduction in the amplitude of the outward K^+ current. Reversibility is illustrated by the recovery trace. (*B*) Parallel time courses of the changes in Po_2 in the chamber and the decrease in K^+ current amplitude. The records shown in *A* are indicated by the corresponding number. Current amplitudes were measured immediately before the end of the depolarizing pulses. External solution as in Fig. 1 with 0.5 μM tetrodotoxin added to block Na^+ channels. Internal solution (inside the patch pipette and the cell) (in millimolars): 80 K glutamate, 30 KCl, 20 KF, 2 $MgCl_2$, 10 Hepes, 10 EGTA, 4 MgATP, pH 7.2). (*C*) Changes in K^+ current amplitude as a function of Po_2 in the bath. Values in the ordinate are normalized and represent measurements (mean ± SD, n = 8) of K^+ current amplitude generated during depolarizations to +20 mV. The line over the data points has been drawn by eye. To facilitate comparison among the various O_2-dependent variables, we have also superimposed the lines correspondings to the plots in Fig. 4 *A* and *B*.

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tial few hundred milliseconds after switching from voltage-clamp to current clamp, increases in response to hypoxia (López-López et al., 1989). However, the modulation of action potential firing by Po_2 over long time periods has not been demonstrated because the membrane potential of dialyzed rabbit glomus cells is not stable and when subjected to current clamp the ionic conductances inactivate in a few seconds. We have circumvented this technical limitation by monitoring for several minutes the action potential firing in intact glomus cells with a cell-attached pipette in which the transmembrane current flow during an action potential is manifested as a biphasic action current signal (Fenwick et al., 1982). The effect of a ramplike exposure to low Po_2 on the electrical activity of an intact, spontaneously active, glomus cell is illustrated in Fig. 6 *A*. The recording configuration and an individual action current at an expanded time scale are shown in Fig. 6 *B*. As suggested by the previous data, the changes in Po_2 are paralleled by gradual and reversible modifications in the firing frequency, which is highest when Po_2 drops below ~70 or 60 mm Hg. A similar pattern of firing frequency versus Po_2 has been observed in three other hypoxic exposures in two different cells.

Differential Inhibition of K^+ and Ca^{2+} Channels by Low Po_2

The results described above clearly indicate that the O_2 dependence of the variables involved in stimulus–secretion coupling in glomus cells is progressively displaced toward the lowest Po_2 levels (see Fig. 5 *C*). Since most of the of Ca^{2+} influx induced by low Po_2 in glomus cells likely occurs during the firing of action potentials, the gradual increase in firing frequency during exposure to hypoxia could explain, at least in part, the delay observed in the building up of an internal Ca^{2+} signal large enough to trigger secretion (see Figs. 1 and 2). However, the fact that in many glomus cells, reductions of Po_2 from 150 to ~70 mm Hg give rise to only tiny, and often unnoticeable, elevations of cytosolic [Ca^{2+}] in spite of a 50% inhibition of the O_2-sensitive K^+ conductance, led us to hypothesize that transmembrane Ca^{2+} influx might be partially prevented during the exposure to moderate hypoxia. Thus, we explored the possibility that voltage-dependent Ca^{2+} channels could be also inhibited by low Po_2. Macroscopic Ca^{2+} and K^+ currents recorded in glomus cells exposed to normoxic and hypoxic solutions are shown in Fig. 7, *A–C*. As reported before (López-Barneo et al., 1988), during depolarizations to +20 or +10 mV (step potential at which we recorded the maximal peak Ca^{2+} current amplitude; see Ureña et al., 1989), low Po_2 produced a selective inhibition of the outward potassium current leaving the inward and the tail Ca^{2+} currents unaltered (see also Fig. 5 *A*). However, during depolarization to a membrane potential of 5 mV, eliciting ionic currents of smaller size, hypoxia led, in addition to the inhibition of K^+ channel activity, to an appreciable reduction in the Ca^{2+} current amplitude. The modulation of K^+ and Ca^{2+} channels by O_2 are not mutually related processes, since our results indicate that they occur independently and in different membrane potential ranges. Fig. 7, *D–F*, illustrates that in the absence of Na^+ and Ca^{2+} channel activity, a fraction of the outward current, perhaps mediated by the Ca^{2+}-dependent K^+ channels, is lost. The remaining K^+ current, representing the activity of the population of voltage-dependent K^+ channels expressed by the cells, was reversibly inhibited by low Po_2. This is an expected result, since the O_2-regulated single K^+ channels recorded in membrane patches of rabbit glomus cells are voltage-gated but independent of cytosolic [Ca^{2+}]. Ca^{2+}-dependent K^+ channels existing in glomus cells are unaffected by low Po_2 (Ganfornina and López-Barneo, 1992*a*). Likewise, Ca^{2+} currents recorded in isolation were also differentially reduced by low Po_2 in cells with the K^+ channels blocked by internal Cs^+ (see Fig. 8).

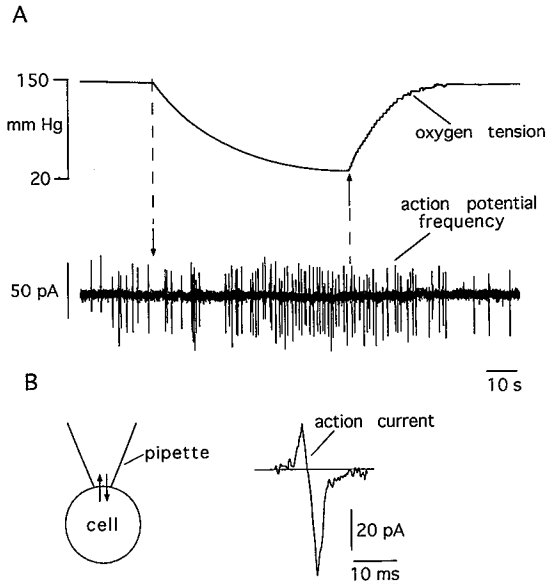

FIGURE 6. Reversible increase in action potential firing frequency during exposure of a glomus cell to low Po_2. (*A*) Firing of action potentials in a cell recorded in cell-attached mode and exposed to hypoxia. (*B*) Recording configuration and time course of an action current at an expanded time-scale. The signal at the right in *B* corresponds to one of the spikes in *A*. External solution as in Fig. 1. Pipette solution as in Fig. 5.

Hypoxic Inhibition of Ca^{2+} Channel Activity Is Strongly Voltage Dependent

The interaction of O_2 with the Ca^{2+} channels was studied after blockade of Na^+ and K^+ channels and using external Ba^{2+} as a charge carrier to increase the size of the current. Superimposed Ba^{2+} current traces recorded

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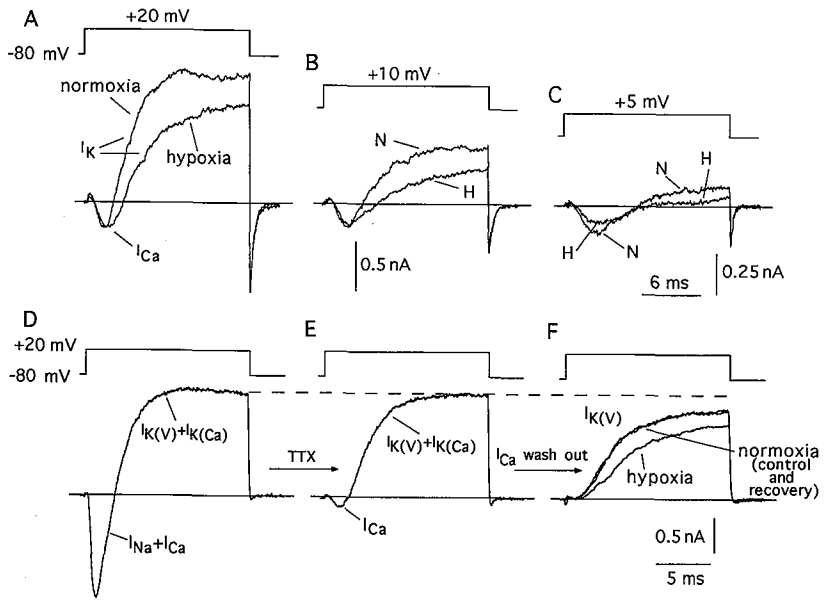

FIGURE 7. Differential inhibition of K^+ and Ca^{2+} currents by low Po_2. (*A–C*) Ca^{2+} and K^+ currents recorded during depolarizations to the indicated membrane potentials. Note that at the three voltages, hypoxia (*H*, $Po_2 \sim 20$ mm Hg) produced inhibition of the outward K^+ current. Hypoxic inhibition of the inward Ca^{2+} current was only appreciated with the depolarization to +5 mV. External and internal solutions as in Fig. 5. (*D–F*) Macroscopic voltage-dependent currents recorded in a glomus cell during depolarizations to +20 mV in normoxic conditions (*N*, Po_2 150 mm Hg). Note in *E* the blockade of the large inward Na^+ current by tetrodotoxin (*TTX*, 0.5 μM) and the washout of the Ca^{2+} channels in cells dialyzed with a solution without ATP (*F*). After the disappearance of both the Ca^{2+} channels and a Ca^{2+}-dependent component of the K^+ current, the remaining outward K^+ current is reversibly reduced in amplitude by low Po_2. Solutions as in Fig. 5 but without MgATP.

in normoxic (Po_2 = 150 mm Hg, *C*) and hypoxic (Po_2 = 20 mm Hg, *H*) conditions during depolarization to the indicated membrane potentials are shown in Fig. 8 *A*. The current-voltage curves in the two experimental conditions are plotted in Fig. 8 *B*. Current amplitude was reduced to ~60% of the control value during depolarization to −20 mV, but no effect was observed at +30 mV. The recovery trace (*R*) at 0 mV illustrates the reversibility of the effect of low Po_2. These data clearly demonstrate that hypoxia inhibits Ca^{2+} channel activity in a marked voltage-dependent manner. A summary of the voltage-dependent effect of low Po_2 on the calcium current recorded in several cells is given in Table I. In the membrane voltage range near action potential threshold (<−20 mV), the inhibitory effect of low Po_2 on Ca^{2+} channel activity is surely more accentuated. However, this could not be studied in detail because of the small size of the macroscopic currents. It is worth mentioning that hypoxia slows the activation time course of the current without an apparent effect on the tail currents representing the closure of the channels (see records at +20 or +30 mV in Fig. 8 *A*). As it occurs with the O_2-sensitive K^+ channels (see Fig. 5 *C*), the hypoxic inhibition of Ca^{2+} channels is also dose dependent and observable when Po_2 is reduced to values immediately <150 mm Hg. In Fig. 9 *A* is shown the time course of Ca^{2+} channel inhibition in response to hypoxia, following roughly the change of O_2 tension in the chamber, as well as the strong voltage dependence of the inhibition of channel activity by low Po_2. This in-

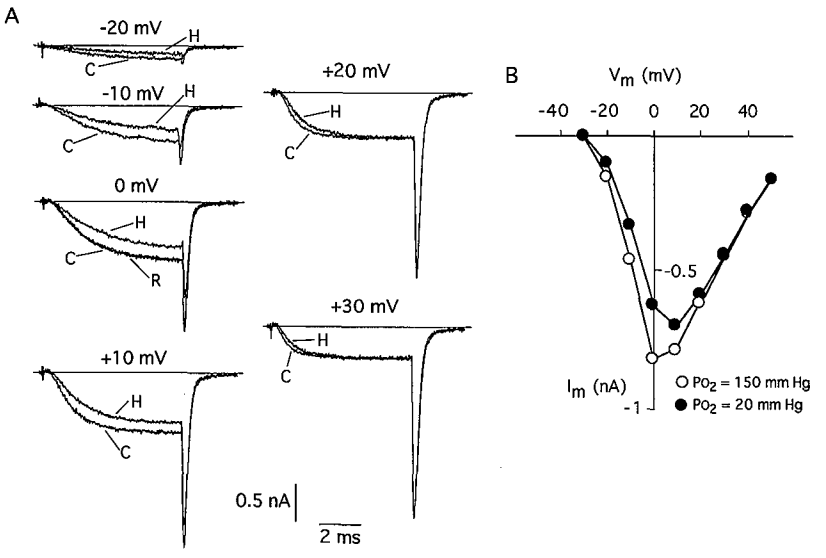

FIGURE 8. Voltage-dependent inhibition of Ca^{2+} channels by low Po_2. (*A*) Barium currents recorded from a glomus cell during step depolarizations to the indicated membrane potentials from a holding potential of −80 mV in control (*C*, Po_2 150 mm Hg) and hypoxic (*H*, $Po_2 \sim 20$ mm Hg) external solutions. Reversibility of hypoxia is illustrated at 0 mV (trace *R*). (*B*) Current–voltage relationship corresponding to the experimental traces shown in *A*. Current was measured immediately before the end of the pulses. Note that the hypoxic reduction of current amplitude is observed only during moderate depolarizations. The recording solutions contained (in millimolars): External (140 NaCl, 2.7 KCl, 10 $BaCl_2$, 10 Hepes, 0.5 μM tetrodotoxin, pH 7.3); internal (100 CsCl, 30 CsF, 2 $MgCl_2$, 10 Hepes, 10 EGTA, 4 MgATP, pH 7.2).

TABLE I

Voltage-Dependent Inhibition of Ca^{2+} Channel Activity by Low Po_2

	Membrane Potential (mV)					
	−20	−10	0	+10	+20	+30
Percentage reduction in peak current amplitude	30 ± 10 (6)	24 ± 7.3 (11)	15.3 ± 6 (10)	9.6 ± 7.1 (10)	6.8 ± 4.8 (6)	2 ± 2 (3)

Current was measured during 15–20 ms depolarizing pulses to the indicated membrane potentials from a holding potential of −80 mV. Low Po_2 was obtained by switching from an external solution equilibrated with air (Po_2 = 150 mm Hg) to another bubbled with N_2 ($Po_2 \sim 20$ mm Hg). Values are given as the mean ± SD and the number of cells in parentheses.

hibition is clearly observable when the membrane potential of the test pulse was −10 mV (*dots*) but negligible in membrane currents generated by depolarizations to +30 mV (*open symbols*). The average Ca^{2+} channel activity–Po_2 relation is shown in Fig. 9 *B* with measurements at two different membrane potentials: −10 mV (*solid symbols*) or +30 mV (*open symbols*). Note that the dose-dependent modulation of the current seen with moderate depolarizations is almost totally absent at positive membrane potentials. Therefore, low Po_2 inhibits Ca^{2+} channel activity when the membrane potential is negative or slightly depolarized but the inhibition is relieved if the membrane is depolarized to potentials more positive than +20 mV. This is a property of the O_2-regulated Ca^{2+} channels that, as discussed in the next section, might have a fundamental functional relevance.

DISCUSSION

In this work we have studied the major variables involved in stimulus–secretion coupling in glomus cells. The simultaneous monitoring of cytosolic Ca^{2+} and dopamine release in single cells has allowed us to analyze in detail the characteristic features of their secretory response to hypoxia. The results demonstrate that in glomus cells both K^+ and Ca^{2+} channel activity are independently inhibited by hypoxia over a broad range of O_2 tensions. The properties and differential effects of O_2 on these ionic conductances help to explain why glomus cells secrete predominantly at low Po_2 values. These data provide a conceptual framework for a global understanding of sensory transduction in the carotid body at the cellular level.

Hypoxia-induced Dopamine Secretion and the Input–Output Relations in Glomus Cells

During exposure to hypoxia, glomus cells respond with a rise of cytosolic [Ca^{2+}] and secretory activity that appears to have two distinguishable phases. An initial period (when Po_2 drops from 150 mm Hg to ~70 mm Hg) is characterized by a small, often negligible, increase of cytosolic Ca^{2+}, which rarely evokes secretory activity. At lower Po_2 values, most cells exhibit a larger and sharper rise of Ca^{2+}, proportional to the severity of the hypoxia, which triggers a burstlike episode of dopamine release. This characteristic response to hypoxia results in hyperbole-like relationships between either internal [Ca^{2+}] or the secretory activity as a function of environmental Po_2. With minor differences, these relationships are comparable with those described for the Po_2 dependence of dopamine release in

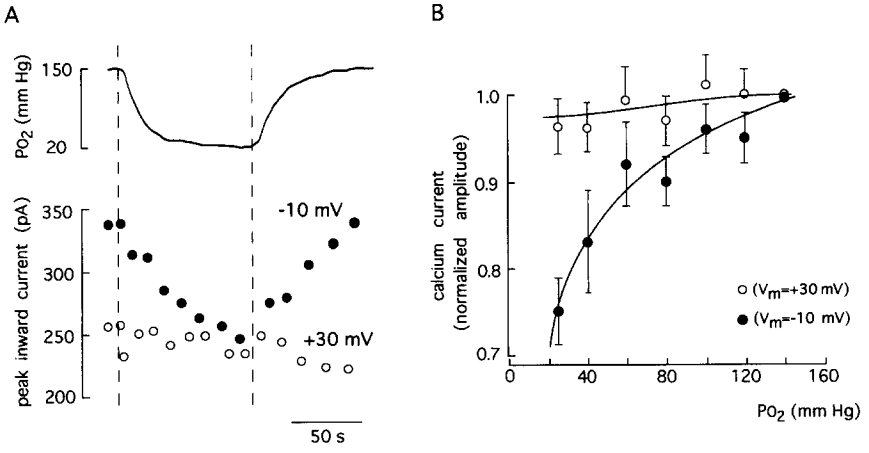

FIGURE 9. (*A*) Time course of the inhibition of Ca^{2+} channel activity by low Po_2 at two different membrane potentials. Pulses of 10 ms where applied from a holding potential of −80 mV. Time between the vertical discontinuous lines is 94 s. (*B*) Dose-dependent inhibition of Ca^{2+} channel activity by low Po_2 and the effect of the membrane potential. Data points are average (mean ± SD, n = 4 cells) measurements of inward calcium currents recorded at two different membrane potentials in cells exposed to various Po_2 levels. Lines over the data points are fitted by eye. Solutions in *A* and *B* are as in Fig. 8. Note that the reduction of Ca^{2+} channel activity by hypoxia is only observable with moderate depolarizations.

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the carotid body or the discharge rate in the afferent fibers of the sinus nerve (Hornbein, 1968; Biscoe et al., 1970; Rigual et al., 1986; Donnelly, 1993; Lahiri et al., 1993). Therefore, the input–output relations in single glomus cells are the same as those of the carotid body; hence, the properties of individual cells can explain the physiologic responses of the entire organ. The appearance of two phases in the secretory response of glomus cells to low Po_2 is mainly a result of the requirement of a minimal cytosolic [Ca^{2+}] to trigger secretion. The estimated threshold level in our experimental conditions (~180 nM) is close to values estimated in other neurosecretory cells (see, for example, Peng and Zucker, 1993). Naturally, these are average values of cytosolic [Ca^{2+}] estimated in intact, fura-AM–loaded cells that do not actually correspond to the [Ca^{2+}] in the vicinity of the membrane required for exocytosis, which is likely in the range of tens of micromolar (Heidelberger et al., 1994; for a review see Burgoyne and Morgan, 1995). As in chromaffin (Chow et al., 1992) and pituitary (Thomas et al., 1993) cells, the lag period between Ca^{2+} entry and exocytosis in glomus cells is of several tens of milliseconds (Ureña et al., 1994), indicating that few secretory granules are docked tightly to the membrane and that most of them are in a non-immediately releasable pool. The threshold may then represent the rise of [Ca^{2+}] in a broad region of the cytosol necessary for recruiting undocked vesicles to the releasable pool (see Burgoyne and Morgan, 1995). Moderate exposures to low Po_2 seem to produce in glomus cells only small increments in action potential firing frequency; thus, the action of immobile cytosolic buffers and the Ca^{2+} uptake and extrusion mechanisms of the cells perhaps contribute to maintain cytosolic [Ca^{2+}] below the secretory threshold. However, it is most likely that the building up of a robust Ca^{2+} signal is mainly prevented by the inhibition of Ca^{2+} channel activity by low Po_2. As discussed below, this could explain why in many glomus cells cytosolic [Ca^{2+}] is kept near the resting value during exposure to moderate hypoxia although a sharp and large rise of Ca^{2+} is observed when Po_2 decreases further.

Oxygen-Sensitive Ca^{2+} Channels

We describe here a previously unknown property of glomus cells, which is the inhibition of Ca^{2+} channel activity by low Po_2 (see Montoro and López-Barneo, 1995). Because the interaction of O_2 with the Ca^{2+} channels is strongly voltage dependent, when the macroscopic Ca^{2+} and K^+ currents are recorded simultaneously using test voltages over 0 mV, the hypoxic inhibition of Ca^{2+} channels is not obvious. This is the reason why we, and other investigators, did not previously notice the influence of O_2 tension on the Ca^{2+} channels and proposed the effect of hypoxia to be highly selective for K^+ conductance (López-Barneo et al., 1988; Delpiano and Herscheler, 1989; Stea and Nurse, 1991). The detailed account of the effect of hypoxia on the properties of the Ca^{2+} channels will be given elsewhere, but it must be mentioned that many of its characteristic features (voltage-dependent inhibition, slowing of activation time course, and lack of effect on channel deactivation) are qualitatively similar to those described for the O_2-sensitive K^+ channels of glomus cells (Ganfornina and López-Barneo, 1992*b*). We have also recently reported the existence of O_2-sensitive Ca^{2+} channels with identical properties in arterial myocytes, where they may contribute to hypoxic arterial relaxation (Franco-Obregón et al., 1995). Thus, it seems that the O_2-sensitivity of ion channel activity is a phenomenon more general than previously thought perhaps because of the existence in all these channel types of a common O_2-sensing domain that influences in a similar manner channel gating (see López-Barneo, 1994).

Differential Hypoxic Inhibition of Ca^{2+} and K^+ Channels and Chemotransduction in Glomus Cells

Although O_2-regulated Ca^{2+} and K^+ channels share many qualitative properties, their differential regulation by O_2 probably determines the specific response pattern of glomus cells to changes in Po_2. The two channel types are regulated over a similar range of O_2 tensions, but the extraordinarily marked voltage dependence of the interaction of O_2 with Ca^{2+} channels converts them into voltage-dependent switches for Ca^{2+} entry free from inhibition only at positive membrane potentials. This property is perhaps important to ensure that during exposure to hypoxia, Ca^{2+} entry is not triggered by small fluctuations of the membrane potential but Ca^{2+} influx is limited to the time period during which the cells are able to generate repetitive action potentials of large amplitude. Thus, during exposure to mild hypoxia, Ca^{2+} entry is most likely precluded by the inhibition of the channels; however, at very low Po_2 values the protracted and severe reduction in the K^+ conductance leads to a maintained high-frequency burst of action potentials, which results in disinhibition of the Ca^{2+} channels and a surge of Ca^{2+} entering the cell. The interrelation between these two variables (membrane voltage and O_2 tension) could then explain how molecules sensitive to O_2 tensions near the atmospheric level can be used in a system evolved to discriminate among lower Po_2 values, such as those of arterial blood and of functional relevance for the regulation of respiration.

In conclusion, sensory transduction in the carotid body is mainly the result of the responses of individual glomus cells to low Po_2. The major steps of chemotransduction in these cells can be explained by a simplified scheme that qualitatively works as follows. Changes of

O_2 tension are detected by O_2-sensitive K^+ and Ca^{2+} channels. Inhibition of Ca^{2+} channels by hypoxia limits Ca^{2+} entry until Po_2 is low enough that the parallel reduction in K^+ conductance elicits a burst of action potentials, disinhibition of Ca^{2+} channels, and a surge of Ca^{2+} influx. The subsequent suprathreshold rise of cytosolic Ca^{2+} triggers transmitter release, which activates the afferent fibers of the sinus nerve. The carotid body has a complicated structure; thus, this basic sequence of events is surely tuned by paracrine interactions among its constituents, which may play a role in adaptations of carotid body function to changes in physiologic demands or in the environment.

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