Oxygen Sensing by Ion Channels and Chemotransduction in Single Glomus Cells

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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 \(P_{O_2}\) produced a rise of cytosolic \([Ca^{2+}]\) with two distinguishable phases: an initial period (with \(P_{O_2}\) 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 \(P_{O_2}\) 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 \pm 43 nM. The results demonstrate a characteristic relationship between \(P_{O_2}\) and transmitter secretion at the cellular level that is comparable with the relation described for the input (O2 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 \(P_{O_2}\). \(Ca^{2+}\) channel activity is inhibited by hypoxia, although in a strongly voltage-dependent manner. The average hypoxic inhibition of the calcium current is 30\% \pm 10\% at \(-20\) mV but only 2\% \pm 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 \(P_{O_2}\) 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 (\(P_{O_2}\)) 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 \(P_{O_2}\) (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, 1992a). 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 \(P_{O_2}\) 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 \(P_{O_2}\) depends on \(Ca^{2+}\) entry through volt-
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 O₂-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 Po₂ 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 O₂-sensitive K⁺ channel activity in dispersed cells is produced when Po₂ 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 Po₂ reaches values << 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 (O₂-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 [Ca²⁺], or dopamine release with Po₂ in the surrounding extracellular milieu. We report here that the relationship between Po₂ 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 O₂-sensitive Ca²⁺ channels in glomus cells whose activity is inhibited by low Po₂ in a strongly voltage-dependent manner. The combined differential inhibition of K⁺ and Ca²⁺ channels by hypoxia helps to explain why the secretory response of the cells is displaced toward Po₂ 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(1-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 (Po₂ ~ 150 mm Hg) or mixtures of N₂ and air to obtain the desired O₂ concentrations. Po₂ in the chamber was monitored with an O₂-sensing electrode (Ganfornina and López-Barneo, 1992a). 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 [Ca²⁺]**

Cytosolic [Ca²⁺] was estimated in unclamped cells loaded with fura-2 by incubation for 30 min at 37°C with saline containing 1 μM fura-2 acetoxyethyl ester. The external solution contained (in millimolars): 140 NaCl, 2.7 KCl, 2.5 CaCl₂, 1 MgCl₂, 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 [Ca²⁺] (Toledo-Aral et al., 1993). Calibration of the fluorescence signals in terms of [Ca²⁺] was performed in vitro as described elsewhere (Gryniewicz 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 (Urefia et al., 1994). The three signals recorded in parallel (fluorescence, amperometry, and Po₂ 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 Po₂ because of Ca²⁺ influx through voltage-dependent channels. This secretory response to hypoxia is illustrated in Fig. 1 with recordings from an isolated,
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; Urefia 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 \(P_{O_2}\). These recordings indicate that during the slow switching from normoxia (\(P_{O_2} = 150 \text{ mm Hg}\)) to hypoxia (\(P_{O_2} \sim 20 \text{ mm Hg}\)) 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 \(P_{O_2}\) 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 \(P_{O_2}\) reaches values near \(\sim 50 \text{ mm Hg}\) (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 \(\sim 150-200 \text{ nM}\). 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 \(\sim 60 \text{ nM}\) and the apparent \([Ca^{2+}]\) threshold required for the initiation of a clear episode of dopamine release was \(180 \pm 43 \text{ nM}\) (mean \(\pm SD\), \(n = 5\) 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 \(P_{O_2}\). 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 \(P_{O_2}\) 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 \(P_{O_2} < 150 \text{ mm Hg}\). These recordings again show the characteristic response to hypoxia described above, which appeared in different experiments with quantitative varia-

![Figure 1](image1.png)

**Figure 1.** Secretory response of single glomus cells to low \(P_{O_2}\). 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. 4B 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](image2.png)

**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.
tions. Fig. 3 A demonstrates that, as shown in Fig. 1 with the ramp-like hypoxic exposures, large changes of cytosolic \([\text{Ca}^{2+}]\) are only observable with PO\(_2\) levels of \(<70\) mm Hg, and that below this PO\(_2\) value, the rise of internal \([\text{Ca}^{2+}]\) 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 \([\text{Ca}^{2+}]\) only increased up to \(-150\) nM. The abrupt suprathreshold increase of \([\text{Ca}^{2+}]\) (up to near 1 \(\mu\)M) 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 \([\text{Ca}^{2+}]\), 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 ramp-like 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 \([\text{Ca}^{2+}]\) 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 hyperbola-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 \([\text{Ca}^{2+}]\) 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 ramp-like 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
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 O₂-sensitive K⁺ channels in response to low Po2 is presumed to be the initial step in the sequence of events leading to the rise of cytosolic Ca²⁺ 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 Ca²⁺ on exposure to hypoxia depends on Ca²⁺ entry through voltage-dependent Ca²⁺ channels (Buckler and Vaughan-Jones, 1994; Urefía et al., 1994). In rabbit glomus cells, the rise of cytosolic Ca²⁺ in response to hypoxia is abolished by either removal of external Ca²⁺ or the application of organic Ca²⁺ channel blockers (Urefía et al., 1994). However, there are important features of this conceptual scheme not yet studied in detail. For example, the relationship between Po2 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 O₂ 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 Po2 following the same experimental protocol of Fig. 1, thereby allowing us to compare the O₂ dependence of the various steps in the process of chemotransduction.

The macroscopic, voltage-dependent Ca²⁺ 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 Ca²⁺ 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 Po2. A quantitative summary of these experiments is given in Fig. 5 C. In addition to the average K⁺ channel activity-Po2 relationship (n = 8 cells), we have plotted in normalized ordinates the cytosolic [Ca²⁺]- and dopamine release-Po2 curves of Fig. 4 to facilitate the comparison of the various O₂-dependent variables.

Note that whereas large changes of cytosolic [Ca²⁺] and secretion are only observed below Po2 ~ 70 mm Hg, the hypoxic inhibition of K⁺ channel activity is roughly linear in the Po2 range studied and therefore detectable when O₂ tension decreases to just below normoxic values (150 mm Hg).

Because dispersed rabbit glomus cells are able to generate large Na⁺- and Ca²⁺-dependent action potentials repetitively (Duchen et al., 1988; Urefía et al., 1989), it can be expected that the inhibition of K⁺ channel activity by low Po2 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 Po2. (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 Po2 ~ 150 mm Hg to another with a Po2 ~ 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 Po2 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 MgCl₂, 10 Hepes, 10 EGTA, 4 MgATP, pH 7.2). (C) Changes in K⁺ current amplitude as a function of Po2 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 O₂-dependent variables, we have also superimposed the lines corresponding to the plots in Fig. 4 A and B.]

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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 $Ca^{2+}$ 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 $[Ca^{2+}]_i$ 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 $[Ca^{2+}]_i$. $Ca^{2+}$-dependent $K^+$ channels existing in glomus cells are unaffected by low $PO_2$ (Ganfornina and López-Barneo, 1992a). 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.
in normoxic (Po2 = 150 mm Hg, C) and hypoxic (Po2 = 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 Po2. These data clearly demonstrate that hypoxia inhibits Ca2+ channel activity in a marked voltage-dependent manner. A summary of the voltage-dependent effect of low Po2 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 Po2 on Ca2+ 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 O2-sensitive K+ channels (see Fig. 5 C), the hypoxic inhibition of Ca2+ channels is also dose dependent and observable when Po2 is reduced to values immediately < 150 mm Hg. In Fig. 9 A is shown the time course of Ca2+ channel inhibition in response to hypoxia, following roughly the change of O2 tension in the chamber, as well as the strong voltage dependence of the inhibition of channel activity by low Po2. This in-

**Figure 7.** Differential inhibition of K+ and Ca2+ currents by low Po2. (A–C) Ca2+ and K+ currents recorded during depolarizations to the indicated membrane potentials. Note that at the three voltages, hypoxia (H, Po2 ~ 20 mm Hg) produced inhibition of the outward K+ current. Hypoxic inhibition of the inward Ca2+ 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, Po2 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 Ca2+ channels in cells dialyzed with a solution without ATP (F). After the disappearance of both the Ca2+ channels and a Ca2+-dependent component of the K+ current, the remaining outward K+ current is reversibly reduced in amplitude by low Po2. Solutions as in Fig. 5 but without MgATP.

**Figure 8.** Voltage-dependent inhibition of Ca2+ channels by low Po2. (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, Po2 150 mm Hg) and hypoxic (H, Po2 ~ 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 BaCl2, 10 HEPES, 0.5 μM tetrodotoxin, pH 7.3); internal (100 CsCl, 30 CsF, 2 MgCl2, 10 HEPES, 10 EGTA, 4 MgATP, pH 7.2).
TABLE I  
Voltage-Dependent Inhibition of Ca\(^{2+}\) Channel Activity by Low Po\(_2\)

<table>
<thead>
<tr>
<th>Membrane Potential (mV)</th>
<th>Percentage reduction in peak current amplitude</th>
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<tr>
<td>-20</td>
<td>30 ± 10 (6)</td>
</tr>
<tr>
<td>-10</td>
<td>24 ± 7.3 (11)</td>
</tr>
<tr>
<td>0</td>
<td>15.3 ± 6 (10)</td>
</tr>
<tr>
<td>+10</td>
<td>9.6 ± 7.1 (10)</td>
</tr>
<tr>
<td>+20</td>
<td>6.8 ± 4.8 (6)</td>
</tr>
<tr>
<td>+30</td>
<td>2 ± 2 (3)</td>
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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\(_2\) ~ 20 mm Hg). Values are given as the mean ± SD and the number of cells in parentheses.

Inhibition 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. 9B 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.

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}

The average Ca\(^{2+}\) channel activity–Po\(_2\) relation is shown in Fig. 9B 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.

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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 PO₂ is mainly a result of the requirement of a minimal cytosolic [Ca²⁺] 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 [Ca²⁺] estimated in intact, fura-AM-loaded cells that do not actually correspond to the [Ca²⁺] 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 Ca²⁺ 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 [Ca²⁺] 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 PO₂ seem to produce in glomus cells only small increments in action potential firing frequency; thus, the action of immobile cytosolic buffers and the Ca²⁺ uptake and extrusion mechanisms of the cells perhaps contribute to maintain cytosolic [Ca²⁺] below the secretory threshold. However, it is most likely that the building up of a robust Ca²⁺ signal is mainly prevented by the inhibition of Ca²⁺ channel activity by low PO₂. As discussed below, this could explain why in many glomus cells cytosolic [Ca²⁺] is kept near the resting value during exposure to moderate hypoxia although a sharp and large rise of Ca²⁺ is observed when PO₂ decreases further.

**Oxygen-Sensitive Ca²⁺ Channels**

We describe here a previously unknown property of glomus cells, which is the inhibition of Ca²⁺ channel activity by low PO₂ (see Montoro and López-Barneo, 1995). Because the interaction of O₂ with the Ca²⁺ channels is strongly voltage dependent, when the macroscopic Ca²⁺ and K⁺ currents are recorded simultaneously using test voltages over 0 mV, the hypoxic inhibition of Ca²⁺ channels is not obvious. This is the reason why we, and other investigators, did not previously notice the influence of O₂ tension on the Ca²⁺ 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 Ca²⁺ 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 O₂-sensitive K⁺ channels of glomus cells (Canforinia and López-Barneo, 1992b). We have also recently reported the existence of O₂-sensitive Ca²⁺ 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 O₂-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 O₂-sensing domain that influences in a similar manner channel gating (see López-Barneo, 1994).

**Differential Hypoxic Inhibition of Ca²⁺ and K⁺ Channels and Chemotransduction in Glomus Cells**

Although O₂-regulated Ca²⁺ and K⁺ channels share many qualitative properties, their differential regulation by O₂ probably determines the specific response pattern of glomus cells to changes in PO₂. The two channel types are regulated over a similar range of O₂ tensions, but the extraordinarily marked voltage dependence of the interaction of O₂ with Ca²⁺ channels converts them into voltage-dependent switches for Ca²⁺ entry free from inhibition only at positive membrane potentials. This property is perhaps important to ensure that during exposure to hypoxia, Ca²⁺ entry is not triggered by small fluctuations of the membrane potential but Ca²⁺ 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, Ca²⁺ entry is most likely precluded by the inhibition of the channels; however, at very low PO₂ 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 Ca²⁺ channels and a surge of Ca²⁺ entering the cell. The interrelation between these two variables (membrane voltage and O₂ tension) could then explain how molecules sensitive to O₂ tensions near the atmospheric level can be used in a system evolved to discriminate among lower PO₂ 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 PO₂. The major steps of chemotransduction in these cells can be explained by a simplified scheme that qualitatively works as follows. Changes of
O₂ tension are detected by O₂-sensitive K⁺ and Ca²⁺ channels. Inhibition of Ca²⁺ channels by hypoxia limits Ca²⁺ entry until Po₂ is low enough that the parallel reduction in K⁺ conductance elicits a burst of action potentials, disinhibition of Ca²⁺ channels, and a surge of Ca²⁺ influx. The subsequent suprathreshold rise of cytosolic Ca²⁺ 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.

The authors wish to thank Dr. Alfredo Franco-Obregón for comments on the manuscript.

This work was supported by grants of the Dirección General de Investigacion Científica y Técnica (DGICYT) of the Spanish Ministry of Science and Education and the European Community.

Original version received 13 June 1995 and accepted version received 14 August 1995.

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